

Keystone Symposia - Poster Abstracts

Autophagy: From Model Systems to Therapeutic Opportunities (B2)

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POSTER NUMBER: 1001

Elucidating the Role of Rab7 in Blood-Brain Barrier Breakdown in Neurological Disease

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Brain endothelial cells form a paracellular and transcellular barrier to blood-borne solutes via tight junctions and scarce endocytotic vesicles. The blood-brain barrier (BBB) plays a pivotal role in the healthy and diseased central nervous system (CNS). BBB damage contributes to increased CNS influx of serum proteins and immune cells, leading to severe pathological and neurological deficits in both ischemic stroke and multiple sclerosis; yet the cell biological mechanisms of how the paracellular BBB dysfunction occurs in these neurological disorders are not very well understood. We have previously shown by intravital two-photon microscopy, using a transgenic mouse strain in which endothelial tight junctions are labeled with eGFP, that tight junction remodeling and degradation associated with impairment in paracellular barrier function accounts for BBB deficits observed within 24-48 hours after reperfusion in transient MCAO, an animal model for ischemic stroke. In contrast, tight junction dynamic remodeling and degradation occurs early in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis, and is primarily responsible for infiltration of Th17 lymphocytes and serum proteins in the inflamed CNS across a damaged BBB.

How is remodeling and degradation of tight junctions regulated at the BBB in these neurological diseases? The small GTPase Rab7 plays an essential role in protein degradation inside the cell as proteins move from the late endosome to the lysosome. In addition, Rab7 also plays a critical role in autophagy. We have generated mice deficient for Rab7 in endothelial cells (Rab7ECKO) and have examined changes in BBB permeability, neuronal survival and neurological deficits in both animal models for ischemic stroke and multiple sclerosis. We find that Rab7ECKO mice have reduced paracellular BBB permeability following transient MCAO, that correlates with preservation of structural integrity and reduced degradation of tight junction proteins as compared to wild-type littermates. Rab7ECKO mice have a reduced neuronal death in both the sensory and motor cortex and have a moderate protection against ischemic stroke as measured by several neurological tasks. Similarly, Rab7ECKO mice are protected against EAE and show reduced CNS immune cell infiltration compared to control (Rab7^{fl/fl}) mice. These findings suggest that Rab7 regulates TJ protein turnover and degradation responsible for the enhancement of paracellular permeability of the BBB in both animal models for ischemic stroke and multiple sclerosis. Moreover, inhibition of Rab7 activation in endothelial cells may protect endothelial barrier function and reduce CNS damage in many neurological disorders.

POSTER NUMBER: 1002

Unearthing of protein phosphatase(s) that antagonize Atg13 phosphorylation and promote Atg1 activation to trigger TORC1 induced autophagy

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Autophagy is a physiological process that degrades cytoplasmic components or organelle, induced by various cellular stresses maintaining cellular homeostasis by eliminating excess or damaged proteins and organelles. So, it is important to understand how autophagy is regulated. Growth regulator TOR complex 1 (TORC1) is a kinase that constitutively phosphorylates autophagy-related protein 13 (Atg13) normally. Cellular stress inhibits TORC1 and causes Atg13 to be dephosphorylated. Subsequently, the Atg1 complex, composed of Atg1, Atg13, Atg17, Atg29, and Atg31, is formed to initiate autophagy. Thus, the Atg13 dephosphorylation is the initial trigger for autophagy. However, the phosphatase contributing to Atg13 dephosphorylation has not been identified. Therefore, I studied i) to identify the phosphatase(s) that dephosphorylates Atg13, ii) to find out phosphatase(s) that promotes and activates Atg1-complex to initiate autophagy, and iii) to understand the molecular mechanisms of autophagy regulation.

Herein PP2A, the protein phosphatase 2A, is an enzyme that in human is encoded by the PPP2CA gene. The PP2A heterotrimeric protein phosphatase is ubiquitously expressed. I found that PP2A antagonizes Atg13 phosphorylation. Conversely, overexpression of non-phosphorylatable Atg13 suppressed defects in autophagy in PP2A mutant. PP2A-Cdc55 and PP2A-Rts1 required for Atg13 dephosphorylation, Atg1 kinase activation, Atg13-Atg17 interaction, pre-autophagosomal structure (PAS) formation and promotes autophagy induction after TORC1 inhibition. Further, we showed that another protein phosphatase Cdc14, late mitosis progressive and stress-responsive phosphatase, dephosphorylates Atg13 optimally and is required for Atg1 kinase activation, PAS formation, and autophagy induction after TORC1 inactivation as well as essential for sufficient induction of ATG8 and ATG13 expression. Moreover, Cdc14 activation provoked autophagy even under normal conditions. This study identified the novel protein phosphatase(s) antagonizes Atg13 phosphorylation and Atg1-complex formation and promotes TORC1 induced autophagy.

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POSTER NUMBER: 1003

Discovery of novel, small-molecule autophagy inhibitors through phenotypic and target-based approaches

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Autophagy, an evolutionarily conserved catabolic process in which cytosolic content is engulfed, degraded in lysosomes, and recycled, has emerged as a promising pathway for therapeutic modulation to impact numerous human diseases, including inflammatory diseases, neurodegenerative diseases, infectious diseases, and cancer. The role of autophagy in cancer is especially enigmatic. Cancer cells depend on autophagy for survival in hypoxic, nutrient-starved conditions, and they can use autophagy to overcome the stress of treatment with certain chemotherapeutics. Therefore, autophagy inhibition appears to be a promising strategy for cancer therapy. However, in some cancer cell types, autophagy inhibition may lead to an increase in tumor growth, and the considerable genetic diversity of cancer has made it difficult to evaluate autophagy inhibitors in clinical trials due to an incomplete understanding of which cancer types may be sensitive to treatment, and thus what patient population would benefit from this therapy. In addition, the molecules commonly used in these studies are chloroquine derivatives, molecules that inhibit autophagy at a late-stage through non-specific lysosomal accumulation. There is an urgent need to develop selective autophagy inhibitors to accurately evaluate the role of autophagy in human biology and disease. This presentation will describe our recent progress to help address this need through the optimization and implementation of both phenotypic and target-based high-throughput screens to identify novel, small-molecule autophagy inhibitors. Preliminary evaluation of the mechanism and selectivity of these molecules, in addition to toxicity in cancer cell lines to identify biomarkers of sensitivity, will also be presented.

3

POSTER NUMBER: 3031

A myotubularin-related phosphatase that regulates autophagic flux

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Macroautophagy (autophagy) controls cellular catabolism by transporting cytoplasmic cargoes to lysosomes for degradation, and it underlies multiple human disorders. Pioneering work in *Saccharomyces cerevisiae* defined the

core autophagy machinery, but animals possess autophagy regulators that were not identified in yeast. We are investigating autophagy programs in the context of animal development to identify novel regulators of this process. The midgut undergoes a developmentally-programmed cell size reduction that depends on autophagy. We screened for previously uncharacterized autophagy regulators in *Drosophila* intestinal midgut cells and identified a myotubularin-related (MTMR) phosphatase. Clonally-expressed CG3530-RNAi knockdown disrupts both Atg8a and ref(2)p autophagy reporter levels and localization, and CG3530 knockdown cells fail to undergo autophagy-dependent developmental cell size reduction. CG3530 encodes a phosphoinositide (PI) 3-phosphatase that is orthologous to human MTMRs6-8, which are catalytically active MTMRs implicated in cell death, neurodegeneration, and autophagy, respectively. How CG3530 regulates these processes remains unknown, and a mechanistic role for MTMR8 in autophagy remains undefined. We hypothesize that CG3530 negatively regulates autophagy by maintaining appropriate levels of PIs at autolysosomal membranes, thereby mediating the rate of autophagic degradation within cells. In support of this hypothesis, we detect increased Atg8a autophagy reporter levels in feeding larval midgut and fat cells, during a time point at which autophagy levels are typically low. CG3530 RNAi-knockdown cells also accumulate large Atg8a- LysoTracker- and Lamp1-positive structures. The CG3530 knockdown phenotype is conserved; siRNA-mediated MTMR8 knockdown in Cos7 cells phenocopies our findings in fly tissues. Moreover, MTMR8 knockdown produces perinuclear localization, increased size, and decreased numbers of LC3-, LysoTracker-, and Lamp1-positive spots, and inhibiting PI(3,5)P₂ production can partially revert this phenotype. These data indicate that MTMR8 and the MTMR encoded by CG3530 have conserved requirements for autophagic flux.

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POSTER NUMBER: 1005

ULK-mediated phosphorylation of ATG16L1 promotes xenophagy, but destabilizes the ATG16L1 Crohn's mutant

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Autophagy is a highly regulated catabolic pathway that is potently induced by stressors including starvation and infection. An essential component of the autophagy pathway is an ATG16L1-containing E3-like enzyme, which is responsible for lipidating LC3B and driving autophagosome formation. ATG16L1 polymorphisms have been linked to the development of Crohn's disease (CD) and phosphorylation of CD-associated ATG16L1 (caATG16L1) has been hypothesized to contribute to cleavage and autophagy dysfunction. Here we show that ULK1 kinase directly phosphorylates ATG16L1 in response to infection and starvation. Moreover, we show that ULK-mediated phosphorylation drives the destabilization of caATG16L1 in response to stress. Additionally, we found that phosphorylated ATG16L1 was specifically localized to the site of internalized bacteria indicating a role for ATG16L1 in the promotion of anti-bacterial autophagy. Lastly, we show that stable cell lines harbouring a phospho-dead mutant of ATG16L1 have impaired xenophagy. In summary, our results show that ATG16L1 is a novel target of ULK1 kinase and that ULK-signalling to ATG16L1 is a double-edged sword, enhancing function of the wildtype ATG16L1, but promoting degradation of caATG16L1.

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POSTER NUMBER: 1006

Caspase 9 is Essential for Autophagosome Maturation through Regulation of Mitochondrial Homeostasis

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Caspase 9 (Casp9) is a well-known initiator caspase which triggers intrinsic apoptosis. Recent studies also suggest various, non-apoptotic roles of Casp9, including autophagy regulation. However, the involvement of Casp9 in autophagy pathway and its molecular mechanisms are not well understood yet. Here we report the non-apoptotic function of Casp9 in positive regulation of autophagy through mitochondrial homeostasis. Growth factor or amino acid deprivation-induced autophagy activated Casp9, but without apoptotic features. Pharmacological inhibition or genetic ablation of Casp9 decreased autophagy flux, while ectopic expression of Casp9 rescued autophagy defects. Initiation and elongation of autophagosomal membrane biogenesis were intact, but sealing of the membranes and autophagosome maturation were impaired, and the lifetime of the autophagosomes were prolonged by Casp9 knockout. Ablation of Casp9 caused an accumulation of Atg3, and decreased lipidation of the ATG8 family members, most severely GABARAP1. Moreover, mitochondrial morphology was abnormal, and membrane potential and reactive oxygen species (ROS) production were reduced in Casp9 KO cells. Exogenously added H₂O₂ restored autophagy flux and corrected ATG3 level, but failed to rescue mitochondrial defects. Our findings reveal a new regulatory link between mitochondria and autophagy through Casp9, especially for the proper operation of the conjugation system and the complete autophagosome formation.

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POSTER NUMBER: 1007

Designed small molecules for target-specific autophagic clearance

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Small-molecule-based targeted protein knockdown (TPD) strategies including PROTACs are rapidly emerging modalities in drug discovery. Unlike siRNA molecules that reduce protein translation, TPD can decrease a disease-related protein directly by proteasomal degradation.

However, the scope of current TPD techniques has been limited substantially, because only a fraction of harmful intracellular species can be substrates of the proteasomal clearance. Substrates such as toxic protein aggregates, impaired organelles, and intracellular pathogens are removed via another catabolic pathway, autophagy.

Here we describe a novel strategy for targeted-clearance: autophagy-targeting chimera (AUTAC) that employs autophagy for removal of intracellular species. We first found that guanine modification of proteins [S-guanylation, Ito et al., Mol Cell 52: 794 (2013)] works as a standalone tag for autophagic clearance. AUTAC contains a guanine derivative as a degradation tag and a warhead moiety to provide target specificity. As expected from the wide ranged substrates of autophagy, AUTAC was able to degrade mitochondria (mitophagy) as well as proteins. When applied to fibroblasts derived from a Down syndrome patient, AUTAC accelerated both degradation of dysfunctional mitochondria and biogenesis of functional mitochondria. The cytoprotective effect of mitochondria-targeted AUTAC was also evidenced in acute mitochondrial injury.

Canonical autophagy is viewed as a nonselective bulk decomposition system, and none of the current autophagy-inducing agents exhibits useful cargo selectivity. AUTAC opens up a new avenue for research on autophagy-based drug discovery with a designed specificity.

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POSTER NUMBER: 1008

Autophagy and lysosome membrane permeabilisation: a novel therapeutic target for psoriasis

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Psoriasis is an inflammatory skin disease characterised by epidermal hyperplasia, enhanced keratinocyte proliferation and altered differentiation. It has recently been shown that autophagy is required for normal epidermal differentiation, and that autophagy is deregulated in psoriatic lesional skin. Dithranol, one of the few topical drugs to induce psoriatic plaque clearance and a period of remission, induces mitochondrial damage and apoptosis in psoriatic keratinocytes *in vivo*; however, the contribution of autophagy to epidermal remodelling and plaque clearance in this context is not known. This research aimed to test the hypothesis that autophagy represents a therapeutic target in psoriasis.

Dithranol activated autophagic flux in primary human keratinocytes as shown by immunofluorescence/Western blotting of LC3 I-II conversion. Damaged mitochondria accumulating in dithranol-treated cells were targeted by the

autophagy proteins p62 and LC3, as evidenced by confocal microscopy and co-localisation with complex II. However, complex II did not co-localise with the lysosome marker Lamp2 in this context, suggesting damaged mitochondria were not degraded by autophagy. Dithranol-induced keratinocyte cell death was partially prevented by combined treatment with Bafilomycin A, a disruptor of lysosomal pH, demonstrated by significantly reduced caspase activity and annexin V staining ($P < 0.05$), and reduced mitochondrial cytochrome c release ($P < 0.01$). In addition, dithranol activated lysosomal membrane permeabilisation (LMP) as shown by release of cathepsins B and D, while specific inhibition of cathepsin B activity partially prevented both dithranol-induced cytochrome c release and loss of cell viability ($P < 0.05$).

Collectively these data indicate that dithranol-induced keratinocyte cytotoxicity is due to both mitochondrial damage and destabilisation of LMP resulting in the enhanced induction of apoptosis. Identification of LMP as underpinning the action of dithranol in keratinocytes may lead to the development of specific strategies for LMP induction and novel therapeutic avenues for the treatment of psoriasis.

POSTER NUMBER: 1009

Influenza A virus triggers a pathway that resembles autophagy but has a distinct molecular basis

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Influenza A virus (IAV) infection causes accumulation of LC3 at intracellular membranes and the plasma membrane. The proton channel activity of the viral M2 protein is necessary and sufficient for this. We provide evidence that IAV-induced LC3-positive intracellular vesicles are not double-membrane autophagosomes, but a highly dynamic single-membrane compartment that exhibits microtubule dependent fusion-fission behaviour. Recruitment of the lipidation complex ATG5-ATG12/ATG16L1 in IAV-induced LC3-lipidation critically depends on the C-terminal WD40 domain of ATG16L1. This domain is dispensable for macroautophagy, but is required for lipidation complex recruitment in response to the M2 proton channel, ionophores and other drugs that raise the pH of vesicles. IAV-induced LC3-lipidation therefore differs from canonical autophagy as it targets single membrane vesicles and relies on a distinct ATG16L1 recruitment pathway. To identify genes involved in this process, we performed whole genome CRISPR knock-out screens. These confirmed that the LC3 lipidation machinery is required, but not the upstream components of canonical autophagy. We identified a GAP complex, hitherto without any reported role in autophagy or related processes, as required for this pathway. Many pathogens cause subtle damage to endomembrane compartments, thereby inhibiting acidification by V-ATPases. We propose that LC3 targeting in this context represents a mechanism for detecting a 'danger' signal of erroneously neutral vesicles.

POSTER NUMBER: 1010

An ATG9A-dependent rescue mechanism compensates RAB18 function in autophagy under lipid-limited conditions

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Autophagy is a highly dynamic degradation pathway that is dependent on an appropriate lipid supply for efficient autophagosome formation. Many cellular compartments or organelles have been suggested as donors, however, the exact regulation of lipid acquisition as well as autophagy network alterations to lipid-limiting conditions remain elusive.

Previously we characterized RAB3GAP1/2 and their downstream target RAB18 as novel positive modulators of macroautophagy and uncovered that these proteins support the acquisition of lipid droplet (LD)-derived lipids for autophagosome formation.

Here, we show that the stable knockout of RAB18 as well as RAB3GAP1 results in an altered LD phenotype; the loss of RAB18 function interferes with LD metabolism, causing an impaired fatty acid release, which in turn results in reduced lipid availability for autophagosome formation. This impact on autophagy provokes adaptive adjustments of

the autophagy network, including increased ATG2B expression and ATG12-ATG5 conjugate formation as well as enhanced ATG2B and ATG9A phosphorylation. Phosphorylation of ATG9A directs the transmembrane protein to the autophagic pathway and this modification is sufficient to rescue basal autophagy in the absence of RAB18. Yet, it is incapable of enabling increased autophagic activity under inductive conditions, which demand an increased lipid supply. Thus, we define the molecular role of RAB18 in connecting LDs and autophagy, emphasize the significance of LD-derived lipids for autophagosome formation, and characterize an ATG9A phosphorylation-dependent autophagy rescue mechanism as an adaptation to limited lipid availability.

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POSTER NUMBER: 1011

Autophagy modulators enhance immunoregulatory properties of MIAMI cells

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Mesenchymal stromal cells (MSCs) are adult stem/stromal cells that can be utilized in various therapeutic applications, including tissue regeneration. MSCs can engage in direct cell-cell contacts and secrete soluble vesicles to facilitate tissue regeneration via multiple mechanisms, including immunomodulation. During the process of harvesting, growing and injection into patients, MSCs are “stressed” which may affect their therapeutic properties. Unfortunately, there's limited information on how upregulation of cellular stress-response mechanisms affect MSC-based therapies. Autophagy is a catabolic stress-response mechanism which upregulates in response to a variety of “stressors”, including starvation; autophagy is also involved in immune responses by contributing to both antigen presentation and secretion of immunomodulatory molecules. We hypothesize that autophagy may also play a role in regulating MSCs' immunomodulatory properties. To test this hypothesis, we utilized MSC precursor cells called “marrow-isolated adult multilineage inducible” (MIAMI) cells which are isolated from cadaver bone marrow. To determine how autophagy modulators affect MIAMI cell's response to inflammation, we stimulated the cells with IFN γ and, in parallel, we either stimulated or inhibited autophagy using tamoxifen (TX) or chloroquine (CQ), respectively. The cells then underwent RNA sequencing (RNA-seq) to determine the effects of TX or CQ co-treatments on cellular response to IFN γ on a molecular level. RNA-seq data indicate that the co-treatments change both mRNA and protein levels of genes responsible for immunoregulatory properties of MSCs. For example, increases in mRNA levels of IDO 1 and 2, HLA-G and CD274 and decreases in mRNA levels of MHC-I and MHC-II were observed with co-treatments. CQ and TX co-treatments also induced expression of a number of long non-coding RNAs as well as several microRNAs targeting the mRNA of HLA genes which contributed to decreases in their levels. Furthermore, while IFN γ treatment increased the surface expression of PD-L1, its expression was further increased with CQ or TX. An improvement in the ability of MIAMI cells to decrease the proliferation of activated T cells was also observed with CQ or TX co-treatments. Altogether, this work suggests potential for autophagy modulators as tools to fine-tune immunoregulatory properties of MSCs.

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POSTER NUMBER: 1013

Using Human Induced Pluripotent Stem Cell Derived Macrophages to Study Xenophagy of *Mycobacterium tuberculosis*

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The *in vitro* study of interactions between the intracellular pathogen *Mycobacterium tuberculosis* (Mtb) and its host macrophage has largely been carried out in mouse macrophages, human monocyte derived macrophages or THP-1 cells. These models have various limitations including differences in bacteria control mechanisms between mouse and human macrophages, genetic abnormalities due to being cancer cell lines, and a lack of genetic tractability of hMDMs. Here we introduce the use of human iPS-derived macrophages (iPSDMs) to probe various aspects of Mtb-human macrophage interactions. We have undertaken a full characterization of the transcriptional response of iPSDMs to Mtb infection through RNA-seq revealing a sustained immune response in Mtb WT but not Mtb Δ RD1 infected macrophages at 48h post infection. Interestingly, we also observed a differential regulation of various

selective and canonical autophagy proteins. Furthermore, we show functional responses including cytokine secretion, Mtb replication and trafficking. Finally, we show these cells to be amenable to overexpression of fluorescent proteins for live cell imaging yielding novel insights into the spatiotemporal interactions of Mtb with the autophagy pathway. Altogether, our data show that human iPSDMs represent a novel and powerful model for studying spatiotemporal dynamics of host-pathogen interactions during Mtb infection.

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POSTER NUMBER: 1014

Understanding the role of p62-mediated selective autophagy in *Drosophila*

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Selective autophagic (SA) degradation of ubiquitinated cargo mediates mitochondrial quality control, combats pathogen infection, and maintains organismal health. Impairment of SA is responsible for neurological disorders such as familial Parkinsonism. In *Drosophila*, SA is mediated through recognition of ubiquitinated proteins and bringing these to autophagic degradation by the SQSTM1/p62 homologue Ref(2)P, the sole known selective autophagy receptor in this animal. UBA (ubiquitin-associated) and PB1 (multimerization)-domain mutants of p62 have been shown to attenuate lifespan in *Drosophila*. Here, we have used the CRISPR/Cas9 system to generate double alanine substitutions in its LIR (LC3-interacting region) motif, which disrupts recognition of the protein with Atg8a/LC3 at the phagophore and thus ideally should inhibit all selective autophagy. Characterization of the LIR mutant flies reveal extensive accumulation of p62-positive aggregates in the larval fat cells and the adult brain, increasing with age. We discovered large scale accumulation of poly-ubiquitinated proteins, indicating defect in SA. The p62 aggregates in the brain are also poly-ubiquitin positive, indicating these are aggresomes- in line with the requirement of p62 in aggresome clearance in mammals. LIR mutant flies have normal lifespan and neuromuscular function, and interestingly, increased ectopic stress tolerance (induced by paraquat feeding). Moreover, we found mitochondrial morphology defects, even though mitophagic flux appeared normal in the mutants under paraquat stress. The data so far indicate- i) aggrephagy defects do not predicate loss in physiological functions and lifespan, ii) possibility of other autophagy receptors in the mitochondria. Further studies along this direction are under way.

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POSTER NUMBER: 1015

Clarifying the regulatory roles of two *Drosophila* myotubularin-related lipid phosphatases, MTMR6 and EDTP, in autophagy

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Autophagy is a highly conserved lysosome mediated self-degradation process of eukaryotic cells. During macroautophagy a double membrane structure forms which sequesters a part of the cytoplasm generating autophagosomes. Autophagosomes then fuse with late endosomes and lysosomes, forming autolysosomes where the degradation takes place. At least two phosphatidylinositol- (PI) derived lipid signalization molecules, phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 3,5-bisphosphate (PI3,5P₂) have significant roles in the autophagic process. Generation of PI3P from PI is catalyzed by PI3KC3/Vps34 complex which is essential for autophagosome formation. PI3,5P₂ takes part in later steps of autophagy. The pattern of these phosphoinositides results from coordinated actions of certain kinases and phosphatases. The latter include myotubularin-related phosphatases, MTMRs, which show substrate specificity towards PI3P and PI3,5P₂ and convert these molecules to PI and PI5P, respectively. Vertebrate genomes encode 16 members of myotubularin protein family, 9 of them are catalytically active. Our group investigated the regulatory role of two active myotubularins, EDTP and MTMR6, in autophagy in the fruit fly *Drosophila melanogaster*. EDTP (egg-derived tyrosine phosphatase) is orthologous to human MTMR14, while *mtmr6/CG3530* is the sole orthologue of human MTMR6, -7, and -8 genes. We performed mutational and RNAi-mediated gene silencing analyses of EDTP and *mtmr6* to uncover their effects on basal and starvation/oxidative stress-induced autophagy in the larval fat body. According to our results, EDTP inhibits basal

autophagy but does not affect the process under stress. In contrast, Mtmr6 promotes basal autophagy in a later step of the process, but inhibits autophagosome formation under stress-induced conditions. These findings reveal that basal and stress-induced forms of autophagy are under different regulatory mechanisms.

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POSTER NUMBER: 1016

AMPK activation through Galectin-9 induces autophagy and affects other processes in response to lysosomal damage

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Autophagy is a cellular degradation pathway that contributes to metabolic homeostasis as well as ensures quality control. AMPK is a known regulator of autophagy and recently we have reported that lysosomal damage activates AMPK. However, the molecular and cellular details of how AMPK activation occurs in response to lysosomal damage and how this in turn modulates autophagy are not known. In the present study, we show that AMPK recruitment to lysosomes is enhanced upon lysosomal damage using the lyso-IP method. Furthermore, galectin-9, which promptly detects lysosomal damage by binding to exposed exofacial glycans, facilitates the recruitment of AMPK to damaged lysosomes. Galectin-9 assists in AMPK α 1 localization since galectin-9 knockout cells fail to activate and recruit AMPK to damaged lysosomes. By using subcellular membrane fractionation we found that following lysosomal damage Lamp-2 shifted from heavier, typically lysosomal membranes to lighter membrane fractions that are positive for LC3-II, along with galectin-9 and AMPK. We interpret this as a shift of lysosomes to mixed-membrane fractions, likely of autophagosomal nature based on LC3-II as a marker. AMPK recruitment was required for this shift of lysosomes to the LC3-II⁺ lighter mixed-membrane fractions, since AMPK knockdown significantly reduced LC3-II movement to Lamp2 positive lighter fractions. Additionally, we investigated whether lysosomal damage caused metabolic reprogramming/shifts in cells as measured by Seahorse Mito Stress test. Our study advances our understanding of how the Galectin-9-AMPK system responds during endomembrane damage by activating AMPK and targeting AMPK to damaged lysosomes. This is an example of how an apex regulator of cellular metabolism, AMPK, which also serves as an upstream regulator of autophagy, becomes engaged and properly targeted to the sites of action where autophagy exerts its quality control function.

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POSTER NUMBER: 1017

Metabolic alterations and accelerated aging in Ambra1 haploinsufficient animals

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AMBRA1 (activating-molecule in Beclin-1-regulated autophagy) is a protein that regulates the early stages of autophagy initiation. In mice, Ambra1 haploinsufficiency (Ambra1^{gt/+}) is linked to several pathological conditions such as Alzheimer's Disease, autism or multiple system atrophy. Here we have investigated the aging phenotype in Ambra1^{gt/+} animals focusing on the nervous system. We observed symptoms of premature retinal aging in Ambra1^{gt/+} adult mice (~15 months). This tendency was increased in geriatric animals and Ambra1^{gt/+} animals displayed significant photoreceptor cell loss, a pro-inflammatory response and decreased night vision. Moreover, the metabolic profile shows important alterations in the nucleotide and glycolytic metabolism in the aged Ambra1^{gt/+} animals. These data indicate that Ambra1 haploinsufficiency results in a premature aging phenotype that is coupled to metabolic alterations.

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POSTER NUMBER: 1018

Fasting Mimicking Diet inhibits autophagy to promote chemotherapy-dependent apoptosis and cancer free

survival in Acute Lymphoblastic Leukaemia model

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One of the new therapeutic strategy to promote cancer free survival is to combine dietary intervention like fasting or fasting mimicking diets (FMDs) with cancer treatments. FMDs enhance the efficacy of a wide variety of therapies ranging from chemotherapy to kinase inhibitors to immunotherapy, weakening cancer cells while protecting normal cells and tissues.

In this work we show that FMD in combination with vincristine (VC), can enhance the death of acute lymphoblastic leukemia (ALL) by inhibition of autophagy and induction of apoptosis by p53 modulation and activation of caspase-3. Our data indicates that a periodic FMD increases the efficacy of VC and extends the cancer free survival of an in-vivo mouse syngeneic ALL model. In vitro, using both murine and human cell lines, we confirm the effect of FMD+VC in inducing apoptotic and necrotic cell death. We identify that the autophagy inhibition is the mechanism used by FMD to potentiate the cytotoxic effect of VC. FMD+VC induces reduction of expression of main autophagic markers as LC3B and beclin1 and increases p62. Furthermore, genetic inhibition of autophagy through knockdown of Atg1/ULK1 and Atg9 impairs leukemia cell growth in vitro, demonstrating the reliance on autophagy for survival. Finally, in vivo treatment of FMD+VC with an autophagy inhibitor shows a synergistic effect on increasing survival in ALL mice.

POSTER NUMBER: 1019

IL-12-mediated intestinal inflammation is regulated by macrophages through autophagy

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Defects in the autophagy pathway is recognized as a contributing factor to inflammatory bowel disease (IBD) pathogenesis. In fact, multiple genes involved in the autophagic pathway are found within IBD risk loci. Autophagy, specifically in intestinal epithelial cells, has proven to be important to maintain intestinal homeostasis. Additionally, an underappreciated role for autophagy in intestinal mononuclear phagocytes has been established but this role in IBD is unclear. Numerous studies confirm that autophagy disruption in myeloid cells during infection leads to excessive inflammation which is associated with upregulated IL-1 and IL-17. This observation is the canonical pathway frequently described linking autophagy dysregulation to excess inflammation. Interestingly, IL-17 is upregulated in IBD; however, the role of IL-17 in IBD pathogenesis has recently been questioned as therapeutic blockage of IL-17 exacerbates inflammation in both IBD patients and animal models of IBD. Thus, there is a critical gap in knowledge and an urgent need to understand how autophagy in macrophages contribute to intestinal inflammation independent of IL-17. Utilizing an animal model of IBD, we show mice with an autophagy defect in myeloid cells were susceptible to intestinal barrier disruption. These mice displayed increased TH1 (IFN- γ) skewing and enhanced colonic IFN- γ levels after barrier disruption with no difference in IL-17 levels in comparison to littermate controls. IL-12p70, is a potent inducer of IFN- γ , therefore we examined if autophagy-deficient macrophages secreted excess IL-12p70. Indeed, we found autophagy is critical for the regulation of IL-12p70 secretion from macrophages. In the absence of the autophagy gene Atg5 macrophages secrete excess IL-12p70 upon TLR and IFN- γ stimulation. Chemical inhibition of autophagy also affected IL-12p70 secretion and intracellular protein levels. In addition, the autophagy receptor, NBR1 but not p62, was critical for IL-12p70 secretion; however, IL-12p70 is not a target for autophagic degradation. Taken together, we reveal autophagy controls TH1/IFN- γ responses in the intestinal microenvironment through the regulation of macrophage-derived IL-12p70.

Using autophagosome size and number to analyze autophagosome biogenesis

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It is imperative for eukaryotic cells to maintain a normal level of autophagic activity - too much has been suggested to cause chemoresistance in cancer cells, while too little has been implicated in the development of neurological disorders. Autophagic activity is defined as the amount of cargo being trafficked to the vacuole/lysosome, which is determined by the size and number of autophagosomes formed. It has been demonstrated that some proteins in the core autophagy machinery (e.g. Atg9) specifically control how many autophagosomes are formed while others (e.g. Atg8) specifically control autophagosome size. We have extended this analysis to additional Atg proteins in order to better understand their role in autophagosome formation. This involves creating mutant yeast strains where the gene of interest is either selectively mutated or put under the control of different non-native promoters, testing for protein levels by western blot, testing for autophagic activity by pho8Δ60 assay, and using transmission electron microscopy to measure the size and number of autophagosomes formed. Using these methods, we found that Atg7 controls both the size and number of autophagosomes. Atg7 is the first enzyme in a ubiquitin-like cascade resulting in the conjugation of Atg8 to PE. Atg8 affects only autophagosome size, while Atg7 affects both autophagosome size and number, suggesting that Atg7 has other roles independent of Atg8 conjugation. The proteins immediately downstream of Atg7 are the E2-like enzymes Atg10 and Atg3; Atg3 conjugates Atg8-PE directly, while Atg10 conjugates Atg12-5 which causes a conformational change in Atg3, activating it as an E2-like enzyme. Therefore, we are studying Atg3 and Atg10 to determine which pathway downstream of Atg7 affects autophagosome number. In addition, we are extending this method to another autophagy core complex by analyzing Atg14, which recruits the PI3K complex to the site of autophagosome formation.

Collagen microencapsulation of human mesenchymal stem cells - An in vitro model for autophagy modulation studies

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Mesenchymal stem cells (MSCs) are adult stem cells derived from clinically feasible sources such as bone marrow and adipose tissues. MSC-based therapeutic strategies have been developed attempting to treat degenerative diseases such as osteoarthritis. However, a critical problem to be tackled before clinical translation is the senescence problem of MSCs. Loss of autophagy is a hallmark of ageing. Deficient autophagy associates with reduced regenerative capacity in aged stem cells. We found that MSCs from aged patients showed poor proliferative potential and rapid ageing. We have previously developed a collagen microencapsulation technology, entrapping hMSCs in nanofibrous collagen meshwork. This leads to the formation of a physiologically relevant 3D microtissue model. Preliminary data showed that when MSCs are microencapsulated, major autophagy markers such as LC3 were significantly downregulated as compared with the 2D monolayer expanded cells. This suggests that the microencapsulation model is more physiologically relevant than the traditional monolayer culture. More interestingly, when we allow the MSCs to transmigrate from the autophagy-inhibited collagen microspheres, upregulation of the major autophagy markers and increased metabolic rate were noted. These data prompt us to use the 3D microencapsulation model as an in vitro model to study autophagy modulation for aged MSCs. This project will impact future development of stem cell based therapy for tissue engineering and regenerative medicine.

Bidirectional control of autophagy by BECN1 BARA domain dynamics

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The class III phosphatidylinositol 3-kinase (PI3KC3) complexes I and II (PI3KC3-C1 and -C2) are central to the initiation and expansion of the phagophore. Membrane targeting of PI3KC3 complexes by their BECN1 subunit is pivotal to their activity and thus pivotal to autophagy. Here, we mapped the interaction of PI3KC3-C2 and its inhibitor Rubicon to the first β sheet of the BECN1 BARA domain by hydrogen-deuterium exchange (HDX) and cryo-EM and corroborated the site with cell-based assays. These data suggested that BARA β sheet-1 unfolds to directly engage the membrane, which was confirmed using protein engineering, giant unilamellar vesicle (GUV) assays, and molecular dynamics (MD) simulations. Using this mechanism, a BECN1 β sheet-1-derived peptide activates both PI3KC3 complexes I and II, while HIV-1 Nef inhibits PI3KC3-C2. These data reveal how BECN1 switches on and off PI3KC3 binding to membranes. The observations explain how PI3KC3 inhibition by Rubicon, activation by autophagy-inducing BECN1 peptides, and inhibition by HIV-1 Nef, are mediated by the switchable ability of the BECN1 BARA domain to partially unfold and insert into membranes.

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POSTER NUMBER: 1021

The Crohn's disease risk factor IRGM limits NLRP3 inflammasome activation by impeding its assembly and by mediating its selective autophagy

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Several large-scale genome-wide association studies genetically linked IRGM to the Crohn's disease and other inflammatory/autoimmune disorders, where the IRGM appears to have a protective function. However, the mechanism by which IRGM accomplishes this anti-inflammatory role remains unclear. Here, we revealed that IRGM/Irgm1 is a negative regulator of the NLRP3 inflammasome activation. We show that IRGM expression, which is increased by PAMPs, DAMPs, and microbes, can suppress the pro-inflammatory responses provoked by the same stimuli. IRGM/Irgm1 negatively regulate IL-1 β maturation by suppressing the activation of the NLRP3 inflammasome. Mechanistically, we show that IRGM interacts with NLRP3 and ASC and hinders inflammasome assembly by blocking their oligomerization. IRGM mediates selective autophagic degradation of NLRP3 and ASC. By suppressing inflammasome activation, IRGM/Irgm1 can protect from pyroptosis and gut inflammation in Crohn's disease experimental mice model.

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POSTER NUMBER: 1023

AIBP regulates macrophage autophagy response to oxidized LDL

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Autophagy plays a crucial role in maintaining homeostasis via the degradation and recycling of cellular components. Autophagy prevents cell death by removal of intracellular damaged organelles and protein aggregates. In previous studies, we have demonstrated that apoA-I binding protein (AIBP) binds to toll-like receptor 4 (TLR4) and surfactant protein B, resulting in inhibition of neuroinflammation and facilitated pain states, as well as acute lung inflammation in mice. Here, we report that AIBP deficiency attenuates oxidized low-density lipoprotein (OxLDL)-induced autophagy in macrophage. OxLDL is a major pathogenic factor in the development of atherosclerosis, the vascular disease that often manifests in myocardial infarction or stroke. Using bone marrow-derived macrophages (BMDM) isolated from wild type and AIBP knockout mice, we demonstrated that AIBP regulates SIRT1-AMPK activation, ROS generation, mitochondrial dysfunction, autophagosome formation, and cell death in response to OxLDL. These results provide new insights into the new function of AIBP in autophagy-mediated

POSTER NUMBER: 1024

A novel role of ATG9A in protection against membrane damage

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We are interested in autophagic and other homeostatic responses to membrane damage. The Atg9 protein in yeast and its mammalian orthologs including human ATG9A are the only integral membrane autophagy core proteins primarily known for their role in autophagosome biogenesis. Since ATG9A traffics through multiple membranous compartments in mammalian cells, we hypothesized that ATG9A might also play a role in cellular responses to lysosomal and plasma membrane damage. We constructed an APEX2-ATG9A proximity biotinylation probe and subjected cells expressing it to LC/MS-MS proteomic analysis following lysosomal membrane damage. For plasma membrane damage, we employed cell surface biotinylation and live imaging, which indicated that ATG9A is retained on or its recruitment is increased to plasma membrane, following mechanical damage of the plasmalemma, its permeabilization using detergents, or the application of plasmalemma pore-forming toxins. Depletion of ATG9A sensitized cells to plasma membrane damage, determined by increased permeability to propidium iodide. We also investigated the mechanisms governing ATG9A recruitment to or its retention at the plasma membrane and how it functions in membrane wound resealing and removal. Thus, we describe a novel role for ATG9A in plasma membrane damage response and homeostasis.

POSTER NUMBER: 1025

Physicochemical Implications of Lysosomotropic Autophagy Inhibitor Efficacy Under Tumor Extracellular Acidosis

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Hydroxychloroquine (HCQ) is a clinically-approved lysosomotropic autophagy inhibitor being investigated for cancer-related autophagy modulation in over 50 clinical trials. Pharmacokinetics (PK) of HCQ have shown a marked disconnect between plasma and tumor concentrations in patient populations, which has been associated with variability in response and consequent inability to determine if therapeutic concentrations are achieved within the tumor. A likely source of this variability can be attributed to the non-uniform acidic microenvironment in solid tumors, preventing HCQ from crossing the cell membrane as it exhibits primarily pH-driven PK with permeability optimized in non-acidic environments. The resulting impact of reduced HCQ membrane permeability due to extracellular (pH_e) acidosis is observed *in vitro* where the range of HCQ IC₅₀ values is shifted from 5-10 μ M in physiologic neutral pH_e (7.2-7.4) to 50-300 μ M in tumor acidic pH_e (6.8). Counterintuitively, cell lines show very little loss of sensitivity to the dimeric cousin of HCQ, Lys05, under tumor acidic pH_e *in vitro* with an increase in IC₅₀ of less than double (3-6 μ M to 5-10 μ M). Differences in potency between the drugs under varying pH_e can be primarily attributed to physicochemical properties of the molecules. PK modeling of *in vitro* conditions based on these properties suggest that Lys05 accumulates in lysosomes more readily than HCQ and is not nearly as influenced by pH_e. Additionally, different cell lines show significantly different levels of HCQ uptake *in vitro* under controlled pH_e - a trend that is observed with the same set of cell lines in tumor-bearing mouse models, though ratiometrically dissimilar to *in vitro*. This indicates the additional contribution of intracellular driving factors of HCQ tumor PK, specifically lysosomal pH and volume fraction. These findings shed light on a primary source of the plasma/tumor PK disconnect observed in cancer clinical trials utilizing HCQ. Additionally, the mathematical relationships between HCQ/Lys05 uptake and their PK driving forces can be scaled to incorporate blood flow and pH gradients, simulating exposure of total tumor and acidic subpopulations in preclinical models.

 POSTER NUMBER: 1026

Selective and non-canonical LC3C-dependent tumor suppressive autophagy targets for lysosomal degradation Postdivision Midbody Rings and Caveolin-1 in renal cancer cells

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Formation of an autophagosome requires insertion into the autophagosomal membrane of lipidated LC3A, B or C. LC3s bind cargo receptors through the LC3-interacting region (LIR) motifs on the receptors, which engage the cargo. LC3C is an evolutionary late gene, present only in higher primates and humans. It is unique in that it evolved into a multifaceted autophagic regulator, significantly more complex than LC3B/A. It maintains a binding site for the canonical LIRs, but it gained a new binding site for LC3C-specific LIR, CLIR. It also has a highly conserved C-terminal 20 amino acid peptide after G126, cleaved in the process of its lipidation. The peptide has a putative proline hydroxylation motif, which is a hallmark for binding of VHL, tumor suppressor in clear cell renal cell carcinoma (ccRCC). This indicates that LC3C can participate in diverse autophagic processes, selectively mediated by different utilization of LIR or CLIR binding motifs, and absence or presence of the peptide. We found that LC3C autophagy has tumor suppressing activity in renal cancer and is induced by VHL (*Mikhaylova et al., Cancer Cell, 2012*). LC3C requires non-canonical preinitiation and initiation complexes, including ULK3 (but not ULK1/2), UVRAG (but not ATG14), Rubicon and PIK3C2A (but not PIK3C3) and TSG101, member of ESCRT. We identified two direct targets selectively degraded by LC3C autophagy: (i) Postdivision Midbody Rings, remnants of midbody structures formed during cytokinesis, the fate of which is under control of stemness. (ii) Caveolin 1, a cholesterol-binding protein of lipid rafts with a well-established oncogenic activity in renal cancer. LC3C C-terminal peptide with an intact proline hydroxylation is necessary for the selection of the cargo. Importantly, VHL interacts with the core autophagy apparatus (ATGs), an indication that it is directly involved in the formation of LC3C autophagosomes. The data point to the new and unique mechanisms of autophagic selectivity.

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 POSTER NUMBER: 1027

A selective ER-phagy exerts procollagen quality control via a CALNEXIN-FAM134B complex

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Autophagy is a cytosolic quality control process that recognizes substrates through receptor-mediated mechanisms. Procollagens, the most abundant gene products in Metazoa, are synthesized in the endoplasmic reticulum (ER), and a fraction that fails to attain the native structure is cleared by autophagy. However, how autophagy selectively recognizes misfolded procollagens in the ER lumen is still unknown. We performed siRNA interference, CRISPR-Cas9 or knockout-mediated gene deletion of candidate autophagy and ER proteins in collagen producing cells. We found that the ER-resident lectin chaperone CALNEXIN (CANX) and the ER-phagy receptor FAM134B are required for autophagy-mediated quality control of endogenous procollagens. Mechanistically, CANX acts as co-receptor that

recognizes ER-luminal misfolded procollagens and interacts with the ER-phagy receptor FAM134B. In turn, FAM134B binds the autophagosome membrane-associated protein LC3 and delivers a portion of ER containing both CANX and procollagen to the lysosome for degradation. Thus, a crosstalk between the ER quality control machinery and the autophagy pathway selectively disposes of proteasome resistant misfolded clients from the ER.

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POSTER NUMBER: 1029

Impaired TFEB-Mediated Lysosomal Biogenesis Promotes the Development of Pancreatitis in Mice and is Associated with Human Pancreatitis

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Impaired autophagy has been implicated in experimental and human pancreatitis. However, the transcriptional control governing the autophagy-lysosomal process in pancreatitis is largely unknown. We investigated the role and mechanisms of transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, in the pathogenesis of experimental pancreatitis. We analyzed autophagic flux, TFEB nuclear translocation, lysosomal biogenesis, inflammation and fibrosis in GFP-LC3 transgenic mice, acinar cell-specific TFEB knockout (KO) and TFEB/TFE3 double knockout (DKO) mice as well as human pancreatitis samples. We found that cerulein activated mechanistic target of rapamycin (mTOR) and increased the levels of phosphorylated TFEB as well as pancreatic proteasome activities that led to rapid TFEB degradation. As a result, cerulein decreased the number of lysosomes resulting in insufficient autophagy in mouse pancreas. Pharmacological inhibition of mTOR or proteasome partially rescued cerulein-induced TFEB degradation and pancreatic damage. Furthermore, genetic deletion of TFEB specifically in mouse pancreatic acinar cells increased pancreatic edema, necrotic cell death, infiltration of inflammatory cells and fibrosis in pancreas after cerulein treatment. TFEB/TFE3 DKO mice also developed spontaneous pancreatitis with increased pancreatic trypsin activities, edema and infiltration of inflammatory cells. Finally, decreased TFEB nuclear staining was associated with human pancreatitis. In conclusion, our results indicate a critical role of impaired TFEB-mediated lysosomal biogenesis in promoting the pathogenesis of pancreatitis.

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POSTER NUMBER: 1030

Starvation-induced and TFEB-mediated endocytosis is essential for MTORC1 signaling and autophagy flux

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The mechanistic target of rapamycin complex 1 (MTORC1) is a central cellular kinase that integrates major signaling pathways, allowing for regulation of anabolic and catabolic processes including autophagy and lysosomal biogenesis. Essential to these processes is the regulatory activity of transcription factor EB (TFEB). In a regulatory feedback loop modulating transcriptional levels of Rag/RRAG GTPases, TFEB controls MTORC1 tethering to membranes and induction of anabolic processes upon nutrient replenishment. We now show that TFEB promotes expression of endocytic genes and increases rates of cellular endocytosis during homeostatic baseline and starvation conditions. TFEB-mediated endocytosis drives assembly of the MTORC1-containing nutrient sensing complex through the formation of endosomes which carry the associated proteins RRAGD, the amino acid transporter SLC38A9, and activate protein kinase B (pT308-AKT). TFEB-induced signaling endosomes en route to lysosomes are induced by amino acid starvation and are required to dissociate TSC2, re-tether and activate MTORC1 on endolysosomal membranes. This study characterizes TFEB-mediated endocytosis as a critical process leading to activation of

POSTER NUMBER: 1031

Novel insights into the tumor suppressor function of *Atg6*

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Beclin-1 is a tumor suppressor gene monoallelically deleted in several cancers. As a core component of the VPS34 PI3K (III) lipid kinase complex, Beclin-1 plays a key role in multiple vesicle trafficking pathways as well as cytokinesis. While its tumor suppressor function has previously been attributed to its role in autophagy, it is not fully understood how impaired Beclin-1 contributes to certain cancers. *Atg6*, the single *Drosophila* orthologue of Beclin-1, shares 71% amino acid identity with the evolutionarily conserved domain of Beclin-1, and 50% overall identity. *Atg6* mutants display defects in autophagy, endocytosis, protein secretion and larval lethality. Similar to *Beclin-1* mutant mice which display hematopoietic malignancies, *Atg6* mutant larvae exhibit enlarged hematopoietic organs, excess blood cells, and blood cell tumor formation.

Using genetic analyses, RNA sequencing data and metabolic profiling, we have identified several cellular phenomena and signaling pathways underlying the hematopoietic defects and lethality associated with *Atg6* mutant animals. *Atg6* mutant larvae launch a systemic and cellular immune response characterized by increased NF- κ B signaling, increased production of lamellocytes, a specialized blood cell type generally only seen in the context of parasitic infection, and a wound healing response. Not surprisingly, we have found that several of the key nutrient sensing pathways active in cancer cells are also elevated in *Atg6* mutant animals. In line with these data, blood cells in *Atg6* mutant larvae undergo entosis, a form of cell competition induced by nutrient restriction involving the engulfment and destruction of live cells by neighboring cells. This mechanism is present in a variety of human tumors, yet it is unclear whether it acts to inhibit or drive tumor progression. Based on these data we believe *Drosophila* to be a powerful *in vivo* system in which to study the role of Beclin-1/*Atg6* in tumor initiation and progression.

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POSTER NUMBER: 1032

Sorting Nexin 5 Functions as a Novel Mediator of Virus-Induced Autophagy and Antiviral Immunity

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Autophagy is an essential component of innate immunity in defense against viral invasion. However, to date, the precise molecular mechanisms underlying virus-induced autophagy remain largely unclear. Therefore, we performed image-based genome-wide siRNA screens to identify cellular factors that are required specifically for virus-induced autophagy, but not for basal autophagy. We identified an endosomal protein, sorting nexin 5 (SNX5), as a novel mediator of autophagy in response to infection with a broad range of viruses, including several medically-important human viral pathogens (such as Zika virus, influenza A virus, West Nile virus, chikungunya virus, Coxsackievirus B3 virus and poliovirus). In contrast, SNX5 is dispensable for autophagy during baseline conditions or in response to nutrient deprivation or mTOR inhibition. We found that SNX5 interacts with components of the PI3KC3 complex, and during virus infection, is required for endosomal generation of PI3P and recruitment of WIPI2. Moreover, *Snx5* knockout mice display increased lethality after infection with both DNA and RNA viruses. These findings suggest a heretofore undescribed role for SNX5 in endosomal PI3KC3 activation, autophagy and antiviral immunity. More broadly, our findings indicate that cells have organellar and stimulus-specific mechanisms for activation of Beclin

POSTER NUMBER: 1033

Inhibiting Autophagosome Turnover Targets Therapy Resistant Acute Myeloid Leukemia Cells Via Metabolic Disruption

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Therapy resistant acute myeloid leukemia (AML) blasts and leukemia stem cells (LSCs) are thought to contribute to re-initiation of leukemia after successful remission causing relapse. The inherent hypoxia of the bone marrow microenvironment reduces chemosensitivity of AML blasts and promotes survival of LSCs; however, the cellular processes that drive this chemoresistance are not well understood. Autophagy is a prosurvival process that allows cells to adapt to a variety of stressors and reduces chemosensitivity in a variety of cancer cell lines. Here, we demonstrate that prolonged exposure of human AML cells to hypoxic oxygen levels typically encountered in the marrow microenvironment (1% O₂) induced autophagic flux. We also show that reducing autophagosome formation using pharmacological inhibitors (MRT68921 or Spautin-1) and genetic knockdowns of Atg7 or LC3B did not enhance chemosensitivity to cytarabine (Ara-C) under hypoxia, suggesting that autophagy is not driving chemoresistance under low oxygen. Blocking autophagic flux and inducing autophagosome accumulation using bafilomycin A1 or chloroquine, however, could significantly overcome hypoxia-induced chemoresistance to Ara-C. We show that bafilomycin A1-mediated inhibition of autophagosome turnover synergized with chemotherapy in vivo and preferentially targeted primary human LSCs. We found that inhibiting late stages of autophagy reduced mitochondrial respiration particularly under hypoxia, suggesting a potential mechanism for LSC targeting by late stage autophagy inhibitors. These findings support lysosome-based autophagy inhibitors as novel therapeutic agents for the treatment of AML.

POSTER NUMBER: 1034

Systematic Analysis of the Mammalian Selective Autophagy Cargo Network

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Autophagy has been recognized as a key factor in the pathogenesis of various diseases, such as cancer, however, the role of selective autophagy, which targets specific proteins for lysosomal degradation, remains poorly defined. This is, in part, due to a lack of knowledge of the comprehensive set of proteins targeted by selective autophagy. Using quantitative organelle proteomics of the autophagy-lysosomal system and gene-editing techniques, we are revealing the selective autophagy cargo network in mammalian cells. Specifically, we have established CRISPR/Cas mediated Knockouts of core autophagy genes (FIP200, ATG5, BECLIN1) as well as 6 unique selective autophagy receptors (OPTN, NDP52, TAX1BP1, TOLLIP, SQSTM1, NCOA4) in various single and combinatorial combinations in a variety of cell lines. We have also established a quantitative TMT-based quantitative proteomic workflow for analyses of whole cell and lysosomal proteome. By comparing biological replicates of wild-type cells to various knockout lines we are measuring the relative abundances of 7000-8000 proteins at the whole cell level as well as 2000-3000 lysosomal proteins per experiment in order to generate a high-confidence map of the mammalian selective autophagy cargo network. The identification of novel cargo will inform our understanding of the role of selective autophagy in the pathogenesis of various disorders.

POSTER NUMBER: 1035

Atg14 protects the Intestinal Epithelium from TNF-Triggered Apoptosis

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The intestinal epithelium is critically important for nutrient absorption and host defense against pathogens. Autophagy factors such as Atg5 and Atg16l1 are required to maintain epithelial cell morphology and function. However, the role of autophagy initiation factors, namely Atg14, within this organ is unknown. This work identifies Atg14 as a factor that protects the intestinal epithelium from cell death. Conditional loss of this gene results in robust apoptosis within the small intestine, leading to villus atrophy and postnatal lethality. Apoptosis is mediated through the endogenous cytokine Tumor Necrosis Factor (TNF). Neutralizing TNF in-vivo or deleting its cognate receptor, Tnfr1, rescues epithelial cell death and restores the villus-crypt structure of the small intestine. Together, this work links a component of the autophagy pathway to a well-characterized inducer of intestinal death and damage.

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POSTER NUMBER: 1036

Tracing autophagosome biogenesis in selective autophagy using correlative light-electron microscopy

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Nascent autophagosomes, also called phagophores, nucleate from a subdomain of the endoplasmic reticulum (ER). We have previously shown using electron tomography that starvation-induced phagophores form membrane contacts with several organelles, including ER, ER exit sites, Golgi cisternae, endosomes/lysosomes, and mitochondria, suggesting that these organelles might play a role in phagophore biogenesis during amino acid starvation. We are currently using correlative light-electron microscopy to investigate phagophore biogenesis in two types of selective autophagy, namely aggrephagy and mitophagy. We aim to clarify the subcellular environment, morphology and possible membrane connections of the selective phagophores at very early stages of formation.

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POSTER NUMBER: 1059

Atg5 Supports *R. australis* Infection in Macrophages *in vitro* and *in vivo*

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Rickettsiae can cause life-threatening infections in humans. Macrophages are one of the initial targets for rickettsiae after inoculation by ticks. However, it remains poorly understood how rickettsiae remain free in macrophages prior to establishing their infection in microvascular endothelial cells. Here, we demonstrated that the concentration of *Rickettsia australis* was significantly greater in infected tissues of *Atg5 flox/flox* mice compared to the counterparts of *Atg5 flox/flox* Lyz-Cre mice, in association with a reduced level of IL-18 in serum. The greater concentration of *R. australis* in *Atg5 flox/flox* bone marrow-derived macrophages (BMMs) compared to *Atg5 flox/flox* Lyz-Cre BMMs *in vitro* was abolished by exogenous treatment with recombinant IL-18. *Rickettsia australis* induced significantly increased levels of LC3-II and LC3 puncta in *Atg5*-competent BMMs, but not in *Atg5*-deficient BMMs, while no p62 turnover was observed. Further analysis found that the co-localization of LC3 with a small portion of *R. australis* and *Rickettsia*-containing double-membrane-bound vacuoles in BMMs of B6 mice.

Moreover, treatment with rapamycin significantly increased the concentrations of *R. australis* in B6 BMMs compared to the untreated controls. Taken together, our results demonstrated that *Atg5* favors *R. australis* infection in mouse macrophages in association with a suppressed production level of IL-1 β but not active autophagy flux. These data highlight the contribution of *Atg5* in macrophages to the pathogenesis of rickettsial diseases.

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POSTER NUMBER: 1037

Cardiac glycosides regulate Beclin 1/Na⁺,K⁺-ATPase interaction during ischemia

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Autophagy is a lysosomal degradation pathway that promotes cellular survival in response to different stress signals such as nutrient deprivation or organellar damage. However, excessive levels of autophagy induction can also be detrimental. One example is autosis, a distinctive type of cell death that requires both autophagy genes and the Na⁺,K⁺-ATPase pump. To further characterize the molecular mechanism of autosis, we performed a genome-wide siRNA screen to identify genes required for autotic death. The top-scoring hit was the alpha subunit of Na⁺,K⁺-ATPase, providing genetic confirmation of the essential role of Na⁺,K⁺-ATPase in autosis. Moreover, autosis-inducing conditions triggered the interaction between Na⁺,K⁺-ATPase and a key autophagy protein, Beclin 1, in a manner that is prevented by cardiac glycosides (inhibitors of Na⁺,K⁺-ATPase activity). These compounds decreased the Beclin 1/Na⁺,K⁺-ATPase interaction in vivo during cerebral ischemia in rats and during renal ischemic injury in mice, in parallel with improving clinical outcomes. Furthermore, endogenous cardiac glycosides controlled the binding of Beclin 1 to Na⁺,K⁺-ATPase during exercise-induced autophagy. Thus, Beclin 1/Na⁺,K⁺-ATPase interaction is a crucial event during autosis and cardiac glycosides block this interaction both in physiological and pathological conditions, preventing autotic cell death and ameliorating ischemic injury.

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POSTER NUMBER: 1064

Defining a Direct Noncovalent Interaction Between an N-terminal Huntingtin Fragment and Ubiquitin

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Huntington's disease is a progressive, late-onset, neurodegenerative disease characterized by involuntary movements, emotional disturbances, and cognitive decline. The mutation that causes Huntington's disease is a poly-glutamine (polyQ) expansion within the Huntingtin protein (HTT). Our lab and others have shown that HTT functions as an autophagic scaffold. PolyQ expansion or knockdown of HTT causes inefficient cargo loading into autophagosomes, leaving an accumulation of empty vesicles and undegraded proteins tagged with Ub (Ub). Many autophagy proteins contain Ub-binding domains (UBDs), however, direct noncovalent binding of HTT to Ub has not previously been demonstrated. Using St14A cells, we previously determined that HTT amino acids 1-502 co-immunoprecipitate with Ub, and that mimicking phosphorylation at S421 (S421D) increases this interaction.

Using GST pull-down analysis, we have now determined that HTT amino acids 232-367 are required for non-covalent direct *in vitro* interaction with Ub. There are several types of Ub chains, each of which is associated with a different subset of cellular functions and signals. We have demonstrated that HTT 232-367 interacts with linear M1 chains (associated with immune signaling and autophagy), K48 chains (associated with proteasomal degradation), and K63 Ub chains (associated with endocytosis and protein trafficking). HTT appears to prefer K48 Ub chains over K63 chains. Most UBDs interact with Ub at its I44 residue; thus, the I44A Ub mutation completely abolishes binding to the most UBDs. We find that HTT 232-367 binds I44A linear Ub, suggesting that HTT may contain one of the few types of UBD that binds independently of I44, such as a Ubiquitin-binding motif (UBM).

We are currently mapping the HTT residues that are important for binding to Ub using mass spectrometry. We also plan to examine the importance of polyQ expansion and of phosphorylation of S421 and S13 on this interaction, using phosphomimetic and phosphoresistant mutations. Finally, mass spectrometry to assess potential ubiquitinated autophagic cargo that may interact with HTT is in progress to better define the types of selective autophagy

scaffolded by HTT.

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POSTER NUMBER: 1038

VAMP7-dependent secretion allows for axonal resilience in growth restricting conditions

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When exposed to fetal growth restriction conditions, the brain is spared of severe damage to a certain extent. Here we show that cultured neurons still grew long axons in nutrient restricted conditions that generate a mild autophagy stimulation. This effect was reproduced by treatments with low doses of rapamycin or Torin-1, which even increased axonal growth. In the contrary, inhibition of autophagy by Spautin led to neurons with multiple axons indicating the need for autophagy for the establishment of neuronal polarity. Mild autophagy stimulated neurite growth in a VAMP7-dependent manner and induced VAMP7 exocytosis in axonal growth cones. VAMP7 and Atg9 were found in small vesicles moving anterogradely along the axon. Proteomics analysis showed that VAMP7 KO induced strong decrease in unconventional secretion. Autophagy induction further stimulated VAMP7-dependent exosome secretion while conventional secretion was not affected. This evidence thus supports the notion that VAMP7-dependent exocytosis contributes to a resilience mechanism to preserve axonal growth in restriction conditions.

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POSTER NUMBER: 1039

Regulation of Human Hematopoietic Stem and Progenitor Cell Fate Determination by TFEB

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Although lysosomes were classically regarded solely as degradative compartments, they are now recognized as key players in many cellular processes. TFEB, a member of the MITF/TFE family, links and coordinates lysosomal biogenesis with different cellular adaptation pathways in response to a variety of stress conditions, including starvation, ER stress and infection. How cells respond to stress and how these programs influence cellular fate decisions are poorly understood, especially in stem cell driven cellular hierarchies. Hematopoietic stem cells (HSCs) drive human blood production and must maintain equilibrium between clearance of damaged HSCs and long-term maintenance of a pool of healthy HSC in the face of homeostatic stressors. Recent studies by us and others show that HSCs, compared to downstream progeny, have a lowered threshold for apoptosis if exposed to increased levels of proteostatic stress but also enhanced prosurvival programs that protect HSCs during homeostasis. We are currently undertaking gain- and loss of function studies of TFEB in human hematopoietic stem and progenitor cell (HSPC) homeostasis and lineage fate determination using colony forming progenitor cell assays (CFC) and xenograft HSC assays. Overexpression of wild-type (TFEB^{wt}) or constitutively active TFEB (TFEB^{S142A}) resulted in a striking increase in granulocyte-macrophage (GM) colonies and a total abrogation of erythroid (Ery) colonies. Conversely, expression of a dominant negative (TFEB^{DN}) increased Ery and reduced GM colonies. Flow cytometry for myeloid (CD33) and erythroid (GlyA) markers substantiated the morphologic CFC assessment. Xenograft assays of transduced HSPCs into mice for 4 weeks confirmed the myeloid lineage bias upon TFEB activation. Thus active TFEB enhances myeloid output while abrogating erythroid lineage and its inactivation results in the opposite phenotype. Collectively, these results uncover TFEB as a novel regulator of cell fate determination during hematopoiesis.

 POSTER NUMBER: 1040

The Pat1-Lsm complex stabilizes ATG mRNA during nitrogen starvation-induced autophagy
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Macroautophagy/autophagy is a conserved catabolic recycling process triggered during nutrient starvation that must be properly regulated to preserve homeostasis. In recent years, transcriptional regulation of autophagy-related (ATG) gene mRNA has emerged as a critical event to induce autophagy when cells are nutrient starved and, conversely, maintain autophagy low levels during nutrient-rich conditions. During starvation, ATG gene transcription is highly induced leading to an increase in ATG mRNA levels, which in turn leads to high Atg protein synthesis and autophagy induction. However, less is known about the post-transcriptional regulation of these ATG mRNAs and the mechanisms behind maintaining mRNA stability during autophagy induction.

Recently, we reported that following nitrogen starvation the mRNA decapping enzyme Dcp2 is inhibited, which prevents exonuclease Xrn1-mediated 5'-3' degradation of multiple ATG transcripts, enabling ATG mRNA accumulation and efficient autophagy induction. However, how ATG mRNA are protected from 3'-5' degradation by the exonuclease complex remains unknown.

The RNA binding protein Pat1/Mrt1 has been proposed as a possible link between 5'-3' and 3'-5' mRNA degradation. Pat1 interacts with the seven Sm-like proteins Lsm1 to Lsm7, which together form the Pat1-Lsm complex; this complex preferentially binds to the 3' UTR of mRNA. The Pat1-Lsm complex has been implicated in protecting the 3' end of mRNA from exosome-dependent 3'-5' degradation.

Using yeast as a model system, we discovered that during nitrogen starvation, the Pat1-Lsm complex binds the 3' UTR of a subset of ATG mRNAs which prevents their 3'-5' degradation by the exosome. During nutrient-replete conditions Pat1 is phosphorylated, which prevents it from binding and protecting the ATG mRNA, thus leading to their degradation and low autophagy levels. Conversely, upon nitrogen starvation Pat1 is dephosphorylated leading to ATG mRNA binding, transcript stabilization, accumulation and robust autophagy induction.

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 POSTER NUMBER: 1041

A Translocation Process for Unconventional Secretion of Interleukine 1-β
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Protein unconventional secretion refers to the delivery of secretory cargoes without a signal peptide out of the cells. The process deviates from the canonical ER-Golgi trafficking route which accounts for the secretion of proteins with a signal peptide. Although the mechanisms for unconventional secretion diversifies, emerging evidence indicates the transition of unconventional secretory cargoes e.g. interleukine 1-β (IL-1β) into a vesicle carrier during secretion and autophagy-related regulation plays an essential role. Because these unconventional secretory cargoes lack a signal peptide, how they enter into the membrane bound vesicles remains a mystery. Our previous work indicates a protein translocation process involved in the entry of IL-1β into a membrane carrier which later merges into the autophagosome trafficking route. In the current study, we identified a single transmembrane protein TMED10 likely to be a translocon for IL-1β translocation into the vesicles. TMED10 is required for the secretion of IL-1β in our reconstituted system, macrophage cell lines, and differentiated macrophage. In vitro reconstitution demonstrates that TMED10 directly facilitates the entry of IL-1β into the vesicles. Fluorescence imaging and electron microscopy indicate that TMED10 colocalizes with IL-1β in a perinuclear tubulovesicular membrane compartment alike to the ER-Golgi intermediate compartment (ERGIC). TMED10 forms an oligomer, which is required for IL-1β association, membrane entry, and secretion. In conclusion, our study suggests that TMED10 may form a protein translocon on the ERGIC which mediates the incorporation of IL-1β into the vesicle.

***In vivo* and *in vitro* studies to investigate the role of autophagy in human tuberculosis**

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Background: *Mycobacterium tuberculosis* (*M.tb*) is one of the world's most successful human pathogens that has infected ~2 billion people worldwide. Host-directed therapy (HDT) is an emerging concept in the treatment of *M.tb* where the host response is modulated to achieve better control of TB. Whilst the induction of autophagy by metformin, BCG and starvation has been demonstrated in peripheral mononuclear cells. This phenomenon in human alveolar cells the site of human disease from persons with varying degrees of susceptibility to TB remains unknown.

Methods: Blood and or broncho-alveolar lavage (BAL) fluid were obtained from participants with varying degrees of *M.tb* susceptibility (presumed latent TB infection, presumed sterilising immunity, previous TB, and recurrent TB). Expression of LC3II protein was quantified by Western Blot and confocal microscopy, and autophagic flux was calculated. Cell cultures were treated *in vitro* with metformin, and starvation media. A subset of the participants received bronchoscopic-instilled BCG with follow-up bronchoscopy at day three; autophagic flux was determined pre- and post-treatment.

Results: Metformin and starvation were potent inducers of autophagic flux in BAL cells {p=0.0008 compared to no metformin n=22 and for starvation p=0.0004 compared to control n=19}. *In vivo*, BCG was an inducer of autophagic flux (p=0.0005 compared to saline control n=13).

Conclusion: These preliminary results demonstrate that metformin, starvation and BCG induced autophagy in human alveolar cells. These findings may have implications for new host-directed therapies against *M.tb*. The next step is to determine if the relevant pathways influence mycobacterial survival *in vitro*.

System-wide profiling of mitotic ageing reveals pro-ageing functions of the autophagy machinery

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Eukaryotic cells undergo a limited number of cell divisions before entering cellular senescence. We have successfully developed a high-throughput system for mitotic ageing in budding yeast using synthetic biology, which selectively and inducibly prevents newborn daughter cells from entering the cell cycle after asymmetric separation from dividing mother cells. As a consequence, cell growth in induced liquid cultures solely depends on the number of mother cell divisions over time and, thus, directly reflects mitotic ageing. We have established fully automated high-throughput mitotic ageing measurements using a newly developed bioinformatics pipeline. Using this platform, we have determined the quantitative effects of each gene in the yeast genome on mitotic ageing under standard conditions at unprecedented resolution revealing a complex and unexpected picture of cellular ageing. Challenging current views, we found that defects in a subset of core autophagy components delayed mitotic ageing by improving mitochondria function revealing novel functional interactions and regulatory roles beyond autophagy flux of the autophagy machinery. We are presenting mechanistic insights into this novel longevity pathway as a new paradigm for the multifaceted nature of ageing.

Novel Phagolysosomal Event Mediated by Autophagic Machinery

The recognition of PAMPs and DAMPs is crucial to the initiation and propagation of inflammation within tissues. However, the majority of PAMPs and DAMPs are unavailable to immune detection as they are located within intact microbes or cells and are typically complexed within stable macromolecular structures that are not in-and-of-themselves immunostimulatory or freely diffusible. Partial digestion of microbes and cells within phagolysosomes of tissue-resident macrophages would potentially generate diffusible PAMP/DAMPs, but our current understanding of phagosomal maturation would have these bioactive molecules sequestered and eventually destroyed within phagolysosomes. By following parameters of phagolysosomes in macrophages in real time using quantitative high-content fluorescent microscopy, we identified a novel process whereby phagolysosomes underwent iterative fusion-fission events with the plasma membrane releasing partially-digested soluble material from the phagolysosomal lumen into the extracellular space. Deficiencies in Beclin1, Atg7, Atg5, PI3K γ and TSC1 significantly decreased frequency of these events in macrophages, while deficiencies in Cybb and Rubicon did not—implicating involvement of canonical autophagic pathways but not LC3-associated phagocytosis (LAP).

Moreover, LC3 showed strong association with the phagolysosomal membrane during and after phagolysosomal-plasma membrane fusion. We have tentatively termed this process eructophagy—from eructare (Latin: to belch), with the suffix -phagy in recognition of the connection with phagocytosis and autophagy. Consistent with a proinflammatory role, we demonstrated that eructophagy is induced with classical activation of the macrophage, the presence of TLR agonists and following contact with live neutrophils. Moreover, we demonstrate the activation of vicinal cells following the release of formyl-peptide-products from the phagolysosome immediately following individual eructophagic events. Together, we propose that eructophagy is a novel process of activated macrophages, that is mediated by canonical autophagic machinery, and which serves to amplify local inflammation through the processing and dissemination of PAMPs and DAMPs.

POSTER NUMBER: 1046

ER-phagy receptors and co-receptors for specific ER turnover

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Selective autophagy represents the major quality control mechanism that ensures the proper turn-over of the cellular organelles. The endoplasmic reticulum (ER) is fragmented and delivered to the lysosome for degradation via a specific type of autophagy called ER-phagy. The recent discovery of ER-resident proteins that bind to mammalian ATG8s has revealed that the selective elimination of ER involves different receptors that are specific for the ER subdomains or ER stresses. FAM134B and RTN3 are reticulon-type morphogen proteins that remodel the ER network and also work as ER-phagy receptors to ensure the basal membranes turnover. Differently, SEC62 and CCPG1 are transmembrane ER receptors that function in response to ER-stress signals.

FAM134B has the intrinsic property to vesiculate ER membranes. The peculiar structure of its reticulon homology domain (RHD) confers to this protein the ability to remodel the ER sheets. In a different way, RTN3 induces ER tubules fragmentation through the oligomerization of its long isoforms, which contains the LIR domains. Disassembly of ER network is a requisite to deliver ER membranes to the lysosomes. For both receptors, the binding to the mATG8s member as well as a functional autophagy flux in the cells are fundamental to fulfil their function as ER-phagy receptors.

We recently discover that the autophagy receptors, in particular FAM134B and RTN3, require the assistance of ER chaperone proteins in order to selectively eliminate specific cargos and accomplish their biological functions. The chaperone Calnexin assists FAM134B and RTN3 in the elimination, via the autophagy-lysosome system, of the misfolded procollagen, which accumulates in the ER lumen. Mechanistically, Calnexin links ER-luminal procollagen with the ER-phagy receptors. FAM134B and RTN3 do not span the ER membrane so they need a co-receptor that could simultaneously binds the procollagen, inside the ER lumen, and the ER-phagy receptors.

POSTER NUMBER: 1047

Mammalian Atg8 proteins regulate autolysosomal biogenesis through syntaxin 16 SNARE complex

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The autophagy pathway terminates in autophagosome fusion with organelles of the endolysosomal pathway to generate autolysosomes where the captured cargo is degraded. Fusion events leading to transformation of autophagosomes into autolysosomes has become a focus of attention; however, a unified understanding of key events is still not fully available. Mammalian homologs of the yeast Atg8 protein (mAtg8s) play a central role in autophagy, but their potential functions in autophagosomal maturation has not been defined. We employed peptide arrays and bioinformatics to identify LC3-interacting regions (LIRs) in the SNARE family proteins, which are known to regulate intracellular membrane fusion and trafficking. A Qa SNARE, syntaxin 16 (Stx16), as well as its cognate Qb and Qc SNAREs, Vti1a and Stx6, all bear LIR motifs upstream of their SNARE domains. Using CRISPR/Cas9-mediated knockout, we confirmed that autophagic flux still continues in a panel of *STX17*-knockout cells, suggesting Stx17 alone is insufficient to drive autophagosome fusion with lysosomes/endosomes. Knockout of both *STX16* and *STX17* blocked autophagic flux as shown by accumulation of lipidated LC3B, as well as decreased mitophagy and pexophagy. In addition, *STX16* and *STX17* double knockout in Keima reporter cell lines significantly reduced the accumulation of Keima proteins in the acidic compartments, in line with reduced autolysosomal biogenesis upon depletion of both STX16 and STX17. Further analysis of the mechanisms revealed that Stx16 is required for the efficient delivery of lysosomal membrane proteins from the *trans*-Golgi network; similar effects were observed in cells knocked out for all six mATG8s (hexa KO). These observations suggest that the Stx16 SNARE complex works together with mAtg8s to mediate autophagic flux by regulating autolysosomal biogenesis.

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POSTER NUMBER: 1048

***Mycobacterium tuberculosis* evades Xenophagy by Cording in the Cytosol of Live Lymphatic Endothelial Cells**

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The ability of *Mycobacterium tuberculosis* to form serpentine cords is intrinsically related to its virulence but specifically how *M. tuberculosis* cording contributes to pathogenesis remains obscure. We show that several *M. tuberculosis* clinical isolates form intracellular cords in primary human lymphatic endothelial cells (hLEC) in vitro and also in the lymph nodes of patients with tuberculosis. We identified via RNA-seq a transcriptional programme in hLEC that activates cellular pro-survival and cytosolic surveillance of intracellular pathogens pathways. Consistent with this, cytosolic access of hLEC is required for intracellular *M. tuberculosis* cording; and cord formation is dependent on the *M. tuberculosis* ESX-1 type VII secretion system and the mycobacterial lipid PDIM. Finally, we show that *M. tuberculosis* cording is a novel size-dependent mechanism used by the pathogen to evade xenophagy in the cytosol of endothelial cells. These results provide a mechanism that explains the long-standing association between *M. tuberculosis* cording and virulence.

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POSTER NUMBER: 1049

Arabidopsis Presenilins are Involved in Autophagy

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Presenilins are the catalytic component of γ -secretase complex which were first identified in the genetic screens of the patients suffering from the Alzheimer's disease. Macroautophagy is a major route which encompasses degradation of the cell components and damaged proteins, lipids and carbohydrates in order to recycle nutrients in response to starvation. Here we report that Arabidopsis presenilin double mutant line *ps1/ps2* shows defective

clearance of autophagosomes in root cells when autophagy is induced through sucrose starvation. In addition to being susceptible to the sucrose starvation, mutant root cells have altered expression levels of important autophagy related genes. Mutant root cells show differential protein accumulations under sucrose starvation leading to inefficient clearance of proteins. Immunoblotting and microscopy techniques combined with autophagy inhibitors such as wortmannin and concanamycin-A suggested aberrant degradation of autophagosomes within the lytic plant vacuoles. In conclusion, our data suggest involvement of the γ -secretase complex and/or presenilins in plant autophagy.

POSTER NUMBER: 1050

The FAF2-VCP complex binds to and regulates ATG9A retrograde trafficking during autophagy

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Autophagy is an intracellular degradation process that plays a vital role in the maintenance of cellular and tissue homeostasis. During autophagy, the multi-spanning transmembrane protein ATG9A shuttles between peripheral reservoirs and phagophores, which reportedly contributes to the expansion of autophagosomal membranes. However, the precise molecular mechanism underlying ATG9A trafficking remains unclear. By using inducible protein crosslinking coupled with affinity purification and proteomics, we identify the ubiquitin-selective segregase valosin-containing protein (VCP/p97), together with its cofactor Fas-associated factor 2 (FAF2), as a novel regulator of ATG9A trafficking during autophagy. Mechanistically, VCP interacts with ATG9A through FAF2. Inhibition of the VCP ATPase activity causes accumulation of ATG9A in LC3-positive vesicles and inhibition of autophagic flux. Consistently, genetic depletion of VCP or FAF2 impairs autophagy under both normal and starvation conditions. Collectively, these findings suggest that the VCP-FAF2 complex plays an important role in the retrieval of ATG9A from autophagosomal membranes during autophagosome biogenesis.

POSTER NUMBER: 1051

LT-IIc, a bacterial type II heat-labile enterotoxin, induces specific lethality in triple negative breast cancer cells by modulation of autophagy and induction of apoptosis and necroptosis

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Despite the recent advances in breast cancer treatment, triple negative breast cancer (TNBC) remains a serious health problem with limited treatment options and poor prognosis. To discover novel therapeutic approaches to treat TNBC, we screened cholera toxin (CT) and the members of the bacterial type II heat-labile enterotoxin family (LT-IIa, LT-IIb, and LT-IIc) for their capacity to induce cell death in TNBC cells. LT-IIc, but not the other toxins, significantly reduced viability of the TNBC cell lines BT549 and MDA-MB-231 (IC₅₀ = 82.32 nM). LT-IIc had no significant cytotoxic effects on MCF10A (IC₅₀ = 2600 nM), a non-tumorigenic breast epithelial cell line, and minimal effects on MCF7 and T47D, ER⁺ breast cancer cells, or SKBR-3, HER2⁺ breast cancer cells. LT-IIc stimulated autophagy through inhibition of the mTOR pathway, while simultaneously inhibiting autophagic progression, as observed by the accumulation of LC3B-II and p62 proteins. Morphologically, LT-IIc induced accumulation of enlarged LAMP2⁺ autolysosomes selectively in TNBC cells. Bafilomycin A1, an inhibitor of autophagic flux, blocked the formation and/or retention of these autolysosomes. The increase in caspase 3, 7 activity and annexin V staining indicated that LT-IIc induced an apoptotic response. Co-treatment with necrostatin-1, however, demonstrated that the lethal response to LT-IIc is elicited, at least in part, by concomitant induction of necroptosis. Collectively, these experiments demonstrate that LT-IIc acts bifunctionally, inducing autophagy while simultaneously blocking

POSTER NUMBER: 1052

RAB7A phosphorylation by TBK1 promotes mitophagy via the PINK-Parkin pathway

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Removal of damaged mitochondria is orchestrated by a pathway involving the PINK1 kinase and the Parkin ubiquitin E3 ligase. Ubiquitin chains assembled by Parkin on the mitochondrial outer membrane recruit autophagy cargo receptors in complexes with TBK1 protein kinase. While TBK1 is known to phosphorylate cargo receptors to promote ubiquitin binding, it is unknown whether TBK1 phosphorylates other proteins to promote mitophagy. Using global quantitative proteomics, we identified S72 in RAB7A, a RAB previously linked with mitophagy, as a dynamic target of TBK1 upon mitochondrial depolarization. TBK1 directly phosphorylates RAB7A S72, but not several other RABs known to be phosphorylated on the homologous residue by LRRK2, *in vitro*, and this modification requires Parkin activity *in vivo*. Interaction proteomics using non-phosphorylatable and phospho-mimetic RAB7A mutants revealed loss of association of RAB7A^{S72E} with RAB GDP dissociation inhibitor and increased association with the DENN domain containing heterodimer FLCN-FNIP1. FLCN-FNIP1 is recruited to damaged mitochondria and this is inhibited in cells expressing RAB7A S72A. Moreover, non-phosphorylatable RAB7A failed to support efficient mitophagy, as well as recruitment of ATG9A positive vesicles to damaged mitochondria. These data reveal a novel function for TBK1 in mitophagy, which parallels that of LRRK2-mediated phosphorylation of the homologous site in distinct RABs to control membrane trafficking.

POSTER NUMBER: 1053

Newly synthesized histone H3 is degraded by chaperone mediated autophagy

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Histones are essential proteins involved in DNA compaction in nucleosomes, chromatin remodeling and gene regulation. Histone, biogenesis, nuclear transport, assembly into nucleosomes and turnover are highly regulated processes assisted by a battery of histone-specific chaperones. Disruption of proper histone biogenesis, trafficking or its accumulation can influence disease development, as described in cancer. Post-translational regulation of protein levels and control of damaged or misfolded proteins are regulated principally by the proteasome-ubiquitin and autophagy-lysosomal protein degradation systems; however little is known about the proteostasis mechanisms or quality control of recently synthesized histones in animal cells. Recently it has been suggested that hsc70 chaperone regulate critical aspects of cytosolic histone biogenesis and regulation of histone protein levels. Hsc70 is a constitutively expressed chaperone involved in the recognition of proteins selectively targeted to lysosomal-dependent degradation by the chaperone-mediated autophagy (CMA) by binding to KFERQ-like sequences. We identified two KFERQ-like CMA-recognition motifs in the protein sequence of histone H3. However, there are no direct evidences of CMA involvement in cytosolic histone degradation or in the regulation of histone biogenesis. Our results show the presence of histone H3 in purified lysosomal fractions of rat liver, and a selective enrichment in hsc70-rich (CMA+) lysosomes by Western Blot analysis. In addition, pulse and chase experiments with selective pharmacological inhibitors of proteasome and lysosome-dependent protein degradation in HeLa cells indicate that a fraction of cytosolic H3 histones is being degraded by lysosomes. We determined direct uptake and degradation of recombinant H3 into isolated rat liver lysosomes confirming that H3 is a CMA substrate. Moreover, using pulse and chase analysis by imaging microscopy of fluorescent tagged H3 we determined localization of H3 into lysosomal compartments in HeLa cells. Additionally, we observed that lentiviral shRNA mediated Lamp2a knockdown in HeLa cells induce an increase in the levels of cytosolic H3 protein, suggesting a role of CMA in the control of cytosolic H3

pools. The cytosolic pool of newly synthesized H3 histone that is destined to the nucleus in association with H4 is stabilized by the chaperone protein NASP. Interestingly, we determined that NASP displaces H3 from hsc70 binding *in vitro* and protects H3 from CMA mediated translocation into isolated lysosomes. In summary, our results suggest a critical role of CMA in the control of cytosolic histone levels and histone biogenesis.

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POSTER NUMBER: 1054

The small non-coding vault RNA1-1 regulates p62-dependent autophagy and aggregate clearance

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RNA-binding proteins represent a decisive layer in the regulation of every aspect of RNA biology. We discovered that the autophagy receptor p62/sequestosome-1 is an RNA-binding protein, prompting us to investigate the physiological significance of its RNA binding. We show that the small, non-coding vault RNA1-1 (*vtRNA1-1*) selectively binds p62 in human hepatocytic cells. Vault RNAs are small non-coding RNA polymerase III transcripts, best known for their association with a giant cytosolic particle termed vault, whose function has remained elusive so far.

We confirmed the specificity of the *vtRNA1-1*-p62 interaction *in cellulo* and *in vitro*, respectively, and identified the RNA-binding interface of p62. We revealed that depletion of *vtRNA1-1* augments, whereas increased *vtRNA1-1* expression restricts, autophagic flux in a p62-dependent manner. Autophagy induced by starvation reduces the levels of *vtRNA1-1* independently of lysosomal activity or p62 degradation, respectively, and starvation results in a progressive decrease in the RNA binding of p62 as well as a decrease in its interaction with *vtRNA1-1*. We observed that p62-dependent ubiquitin aggregate clearance is enhanced in the absence of *vtRNA1-1*. We also characterized RNA-binding mutants of p62 and show that lack of *vtRNA1-1* binding renders the mutants engaging more actively with the LC3B and GABARAP than the wild-type counterpart. Mechanistically, we provide evidence that *vtRNA1-1* interferes with the multimerization of p62, which is in turn crucial for p62 activity.

Thus, the small non-coding *vtRNA1-1* directly inhibits p62's activity in autophagy. These findings uncover an unprecedented example of a "riboregulator" modulating the function of an autophagic protein. We will discuss the implications of our findings on selective autophagy in general.

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POSTER NUMBER: 1055

The role of TFEB in the innate host defence against intracellular bacterial pathogens

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Autophagy is an essential cellular process through which cells recycle constituents including large protein complexes and defective organelles. The autophagic machinery also specifically targets intracellular bacteria, resulting in their delivery to lysosomes and subsequent degradation. This process, also referred to as xenophagy, was shown to be essential for the defense against pathogens such as *Shigella*, *Salmonella* and *Listeria*. These intracellular bacterial pathogens are the causative agents of Shigellosis, Salmonellosis and Listeriosis respectively, which cause global threats for the human population. Recently, the transcription factor TFEB has emerged as a critical regulator of autophagy. However, the role of TFEB in the control and restriction of intracellular bacterial pathogens remains poorly defined. At present, this question has been mainly addressed in *Caenorhabditis elegans* for which the TFEB homolog HLH-30 was found to control innate immune defense through the regulation of antimicrobial and autophagy genes. We hypothesized that TFEB is activated during bacterial infection and is essential for controlling xenophagic responses to intracellular bacteria. Our data indeed demonstrate that the three intracellular pathogens trigger TFEB activation, albeit with different kinetics and profiles. While mTOR inhibition, which also occurs in the course of infection with these pathogens is likely a critical mediator of TFEB induction in infected cells, our data unexpectedly highlight the existence of *Shigella*-specific modes of TFEB regulation, which will be presented at the conference. Using TFEB knockout cells, we will also present results that delineate the

global role of TFEB in xenophagy and transcriptional reprogramming during infection. This study will provide novel insights into the role played by TFEB in controlling innate immunity to intracellular bacterial pathogens.

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POSTER NUMBER: 1056

Omeasome-proximal PtdIns(4,5)P₂ couples F-actin mediated mitoaggregate disassembly with autophagosome formation during mitophagy

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Cells govern their homeostasis through autophagy by sequestering substrates, ranging from proteins to aggregates and organelles, into autophagosomes for lysosomal degradation. One puzzle has been how smaller autophagosomes can engulf larger organelles. Here, we show that during Parkin-mediated mitophagy, the grape-like ubiquitinated mitoaggregates disassemble into smaller pieces via the actinomyosin system to allow engulfment. During mitoaggregate disassembly, we observed spatially-associated, synchronous formation of circular F-actin and BATS-labeled autophagy initiation sites near mitochondria suggesting that F-actin downsizes substrates and drives autophagosome formation. Interestingly, PtdIns(4,5)P₂ instead of PtdIns(3)P regulates this mitophagy-associated formation of circular F-actin and BATS-sites. Selective depletion of PtdIns(4,5)P₂ near omeasomes, the endoplasmic reticulum (ER) subdomains involved in autophagosome formation, impairs mitoaggregate disassembly. Our findings demonstrate that ER subdomain-localized PtdIns(4,5)P₂ is an upstream signaling lipid that coordinates F-actin mediated mitoaggregate disassembly with autophagy initiation during Parkin-mediated mitophagy.

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POSTER NUMBER: 2001

Investigating the roles of *VMA21* & *MTMR3* in relation to autophagy and myopathy with excessive autophagy (MEA)

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It has been established that autophagy is a highly conserved process and is essential in degrading proteins. Dysregulation of this pathway can lead to many different groups of diseases, such as autophagic vacuolar myopathies, specifically myopathy with excessive autophagy (MEA). MEA is a genetically heterogeneous progressive skeletal muscle disease characterized by vacuolated myofibers. In MEA patients, cells have aberrant autophagy, vacuolization or abnormal lysosomal proteolysis. Presently, mutations in the X-linked gene *VMA21* are the only known cause of MEA, but these mutations account for only a subset of MEA cases. It is hypothesized that *VMA21* may play a role in autophagy regulation. By further understanding the roles of these genes, the pathomechanism of MEA can be more defined and potential therapeutics or treatment strategies can be developed. In order to do so, I will establish different MEA disease models using cell lines, zebrafish and mice models. With these different models and patient cells the specific functions of these genes can be elucidated.

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POSTER NUMBER: 2002

Dichotomous role of stromal cell autophagy for cancer progression

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Autophagy is known to suppress tumor initiation by removing genotoxic stresses in normal cells. Conversely, autophagy is also known to support tumor progression by alleviating metabolic stresses in neoplastic cells. To suppress cancer progression, clinical trials have been focused on blocking autophagy through a systemic administration of lysosomal degradation inhibitors. The consequence of this systemic autophagy inhibition, however, is largely unknown. Since myeloid cells play crucial roles in cancer progression, we examined the effect of autophagy gene (*Atg5*, *Atg7*, *Atg14*, *Atg16l1*)-deficient myeloid cells on the progression of autophagy-competent tumors (B16, MC38, MMTV-PyMT). Here we show that blocking autophagy only in myeloid cells is sufficient to affect tumor progression in a context-dependent manner. In a tumor implantation model, the growth of implanted tumors was substantially reduced in mice with autophagy-deficient myeloid cells; T cells infiltrated deeper into the tumors and were responsible for the reduced growth of the implanted tumors in these mice. In an oncogene-driven tumor induction model, however, the induced tumors grew faster and metastasized more in mice with autophagy-deficient myeloid cells. T cell infiltration into the tumors was similar in this case, but tumor-infiltrated myeloid cells were differentially activated. These data demonstrate that autophagy in stromal cells can be critical for tumor progression and can either suppress or promote tumor growth depending on the context. This study warrants a further investigation on the effect of systemic autophagy blocking on tumor progression and development of selective autophagy blocker for effective control of tumors in an organism level.

POSTER NUMBER: 2003

ULK1 regulates central carbon metabolism and drug-sensitivity in leukemic stem cells in autophagy-independent and autophagy-dependent manner

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Leukemic stem cells (LSCs) are of significant clinical importance since current therapies do not target this population. We have demonstrated that autophagy represents one of the main features involved in the survival/persistence of LSCs following front line treatment with tyrosine kinase inhibitors (TKI) in chronic myeloid leukaemia (CML). Therefore, inhibition of autophagy embodies a strategic target for LSCs eradication. Our study, using stem cell-enriched (CD34⁺) patient-derived CML cells, shows that TKI treatment activates ULK1, demonstrated by phosphorylation of its downstream target ATG13. This correlates with induction of autophagy flux, assessed by RFP-GFP-LC3 and degradation of autophagy substrates. Using a CRISPR-Cas9-mediated ULK1 knock-out (KO) we show that TKI treatment fails to induce autophagy in ULK1 KO cells, resulting in 30% increased sensitivity to TKI treatment ($p < 0.001$).

Here we also uncover a new combination treatment exploiting a novel ULK1 kinase inhibitor (ULK1i) that is under pre-clinical development. ULK1i inhibits ULK1-mediated autophagy in a concentration-dependent manner monitored by mitochondrial degradation in Parkin-dependent "enhanced mitophagy" assay. This correlates with increased mitochondrial respiration, measured by oxygen consumption rates ($p < 0.01$), increased levels of mitochondrial reactive oxygen species (ROS), and decreased levels of glycolysis ($p < 0.01$). Interestingly, using autophagy deficient cells (i.e. ATG7 KO) we show that while the effect of ULK1i treatment on mitochondrial respiration is autophagy-dependent, the effect on glycolysis is independent of autophagy.

Of clinical relevance, ULK1i promotes myeloid differentiation of LSCs *in vitro*, potentiating TKI effects in targeting CML stem cell survival ($p < 0.01$), with minimum effect on normal stem cells. In robust patient-derived xenograft model, ULK1i targets human CML CD34⁺133⁺ LSCs *in vivo* when used in combination with TKI treatment ($p < 0.05$). In conclusion, our findings describe a novel metabolic role for ULK1 in LSCs and provide a strong rationale for clinical development of specific autophagy inhibitors as a therapeutic strategy to more effectively target LSCs in CML patients with minimal residual disease on TKI treatment.

POSTER NUMBER: 2004

Using a CRISPR/Cas9 Screen to Find an Autophagy Dependent Cancer Signature in Canine and Human Osteosarcoma

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It has recently been reported that some subtypes of breast cancer use autophagy as their main survival mechanism even in unstressed environments. The autophagy-dependent subtypes preferentially benefited the most from treatment with autophagy inhibitors alone and in combination therapies. However, there is not a good way to assess autophagy dependency in the clinic and it has not been evaluated in other cancer types. Osteosarcoma, a type of bone cancer, is rare in humans. Survival rates have not significantly improved in 30 years and many patients are or become resistant to conventional chemotherapy, potentially in part due to an autophagy-dependent mechanism. Conversely, osteosarcoma is one of the most common cancers in dogs. Canine osteosarcoma (cOSA) has clinical, pathological, and genomic characteristics nearly indistinguishable from the human form of the disease (hOSA), and the much higher incidence of cOSA make it a valuable surrogate for testing therapeutic strategies with potential application to hOSA. In the studies presented here, autophagy dependency was determined in cOSA and hOSA cell lines using three methods: 1) sensitivity of cell lines to a standard of care autophagy inhibitor hydroxychloroquine (HCQ), 2) shRNA knockdowns of autophagy genes, and 3) CRISPR/Cas9 knockouts of autophagy genes. The cell lines showed differential sensitivity to HCQ, with a 10-fold difference between the most sensitive and most resistant cell line. This data was corroborated by shRNA knockdowns of ATG5, ATG7, BECN1, and ATG13; cell lines that were more sensitive to HCQ treatment had more cell death following ATG knockdowns, indicating these cell lines are autophagy-dependent. Knockout data using twelve autophagy genes is currently being collected with a CRISPR/Cas9 assay since it is a more robust and effective system to monitor cell growth and death. Ultimately this data will be used to assess genomic signatures of autophagy dependence to identify the subset of cancer patients that will benefit from autophagy inhibition alone or in combination with other chemotherapies.

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POSTER NUMBER: 2005

Galectin-3 governs and coordinates ESCRT and autophagic responses during endomembrane damage

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Cells respond to endomembrane damage by deploying a concerted set of activities based on ESCRT and autophagy systems as the main mechanisms for maintaining the endomembrane homeostasis. However, whether and how ESCRT and autophagy cooperate in repair and removal of endomembrane damage is not known. Previous studies suggest that these systems may act separately in this context. Galectin-3, a member of the beta-galactoside glycan-binding family of cytosolic lectins, recognizes exposure of luminal/exofacial glycans following membrane damage. Here we show that galectin-3 responds to lysosomal damage and functionally and mechanistically coordinates ESCRT and autophagic responses. At early stage of lysosomal damage, galectin3 recruits Alix, which we show is important to initiate ESCRT system activities for repairing lysosomal damage. Furthermore, galectin-3 promotes the interaction between Alix and CHMP4, the downstream ESCRT-III component. Galectin-3 also regulates the activity of the autophagy system for cleaning the persistently damaged lysosomes. Thus, a novel function of galectin-3 is to participate in and coordinate the response of both the ESCRT and autophagy systems in a sequential homeostatic response to endomembrane damage.

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POSTER NUMBER: 2006

GABAergic signaling linked to autophagy against intracellular bacterial infections

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the central nervous system and has been extensively studied in neurological disorders. The optimal regulation of GABA-glutamic acid circuits is critical for the physiological function of the central nervous system. A growing body of evidence has shown that a variety of peripheral immune cells express functional GABAergic components. Nonetheless, given the potential role

for GABA in the modulation of innate immune responses, it is poorly understood whether and how GABAergic signaling regulates antimicrobial host defenses in the context of bacterial infections. Here we demonstrate that GABAergic activation enhanced antimicrobial responses against intracellular bacterial infection in macrophages and *in vivo*. GABAergic activation promoted autophagy activation, leading to enhanced phagosomal maturation and antimicrobial responses against mycobacterial infection. In contrast, GABAergic inhibition increased bacterial loads in mice and zebrafish, suggesting that the GABAergic defense plays an essential function in metazoan host defenses. Our study identified a previously unappreciated role for GABAergic signaling in linking antibacterial autophagy to enhance host innate defense against intracellular bacterial infection.

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POSTER NUMBER: 2007

Autophagic death of neural stem cells mediates chronic stress-induced decline of adult hippocampal neurogenesis and cognitive deficits

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Autophagy is generally regarded as a cytoprotective mechanism, and it remains a matter of controversy whether autophagy can cause cell death in mammals. Here, we show that chronic restraint stress suppresses adult hippocampal neurogenesis in mice by inducing autophagic cell death (ACD) of hippocampal neural stem cells (NSCs). We generated NSC-specific, inducible *Atg7* conditional knockout mice and found that they had an intact number of NSCs and neurogenesis level under chronic restraint stress and were resilient to stress-induced cognitive and mood deficits. Corticosterone treatment of adult hippocampal NSC cultures induced ACD via serum- and glucocorticoid-inducible kinase 3 without signs of apoptosis. Our results demonstrate that ACD is biologically important in a mammalian system *in vivo* and would be an attractive target for therapeutic intervention for psychological stress-induced disorders.

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POSTER NUMBER: 1061

Lysine demethylase 3B (KDM3B) regulates autophagy via transcriptional activation of target genes

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Autophagy is a self-degradative physiological process, which is critical for maintaining homeostasis and balancing energy sources in response to various stresses, such as nutrient deprivation. It is a highly conserved catabolic process in eukaryotes, and important for the cell survival by degrading unnecessary or excessive cellular components and recycling them as building blocks for the synthesis of essential molecules. Although, the dysregulation of autophagy has been reported to contribute to various diseases including cancers and neurodegenerative diseases, the molecular systems for the epigenetic regulation of autophagy are poorly understood. Here, we found that the level of Lysine Demethylase 3B (KDM3B) increased in nutrient-deficient HCT116 cells, colorectal carcinoma cell line, resulting in activation of the autophagy-related genes. KDM3B enhanced transcription by regulating H3K9me2 on the promoter of these genes. Furthermore, we observed that overexpression of KDM3B induced autophagy in HCT116 cells. Collectively, these data suggested the critical role of KDM3B in the regulation of autophagy-related genes via H3K9me2 demethylation and induction of autophagy in nutrient-starved HCT116 cells.

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Quantitative proteomics reveal stable and dynamic autophagic cargo profiles during postnatal brain maturation

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Postnatal maturation of the mammalian brain is not static, but rather organized into three distinctive stages of synapse formation, elimination and maintenance. Accordingly, the neuronal proteome needs to be dynamic to support these synaptic changes. As the autophagy-lysosome degradation system is a major component of proteostasis, we set out to characterize how this system contributes to brain maturation. To this end, we performed quantitative proteomics in autophagosomes that were biochemically purified during synapse elimination or maintenance. This unbiased approach revealed in total 898 proteins comprising the brain autophagic cargo, which was validated by examining the following three criteria: (1) cargo is accumulated in the autophagy-deficient brain; (2) cargo is differentially abundant in the autophagosomal lumen at different stages and (3) cargo is identified in its final destination for degradation by performing lysosomal proteomics. Our findings reveal that autophagic cargo is mostly stable across brain maturation and consists of proteins implicated in a variety of cellular processes such as synapse maintenance, cytoskeleton, mitochondrial function, endocytosis, trafficking and translation. Intriguingly, we also identify a smaller cargo fraction, which is highly dynamic between the stages of synapse elimination and maintenance and is mostly represented by synaptic and cytoskeletal proteins. This finding unravels the key protein substrates of autophagy whose elimination dictates the homeostasis of the neuronal proteome during key stages of brain maturation.

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Development of a reporter assay for high throughput screening of autophagy in Vici syndrome

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Vici syndrome is a rare congenital disorder of autophagy that presents with multi-systemic involvement and profound neurological impairment including callosal agenesis. Vici patients have a mean survival of 24 months. Vici syndrome is caused by biallelic loss of function mutations in the gene *EPG5*, a key mediator of autophagy. Vici syndrome can present in the neonatal period in the most severe cases, but a number of recent reports in the literature have shown that milder mutations can present in early childhood with an initially milder phenotype, though all forms of Vici show progressive symptoms, particularly in the brain.

To date, there is no treatment for Vici syndrome or any congenital disorder of autophagy, only palliative care and symptom management. Given the degenerative aspect of Vici syndrome phenotypes, drug therapy could manage disease progression and improve patient quality of life. Identification of compounds that may rescue the cellular defect in autophagy is a critical first step in development of drug therapy for Vici syndrome. To this end, we have implemented a dual fluorescence reporter assay in a lentiviral system to monitor autophagy in real time in Vici patient and control cells. We compared this assay with other methods to monitor autophagy in live cells such as baculovirus-based transfection of fluorescent proteins and dye-based methods. Results showed a more robust signal with fluorescently labeled autophagic proteins vs. dye-based methods, despite variable transfection rates with baculovirus. Additionally, we observed a significant increase in number of autophagosomes per cell which occupy a higher percentage of the cell body in Vici patient cells compared to controls. This assay will allow for real-time monitoring of autophagy in live cells for multiple downstream applications, including high-throughput drug screening of our Vici patient cells.

 POSTER NUMBER: 2011

DRAM-4 and DRAM-5 as a novel regulators of cell death and autophagy

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Recently we identified two new proteins related to DRAM-1 (Damage-regulated autophagy modulator-1). We have named these proteins DRAM-4 and DRAM-5, with DRAM-5 existing in two distinctive isoforms, DRAM-5A and DRAM-5B. The mRNA of DRAM-4 and 5 can be detected in various cancer cell lines with high abundance in breast cancer cells. At the cellular level, DRAM-4 localizes to early endosomes while DRAM-5 can be found in adherens junctions. Despite the differences in localization, the intracellular levels of DRAM-4 and 5 seem to be connected as the deletion of DRAM-4 leads to elevated levels of DRAM-5 and vice versa. Such a compensatory effect, may suggest a collaboration between DRAM-4 and 5 in executing their cellular functions.

Since the other DRAM family members play a well-established role in the regulation of cell death and autophagy, we investigated whether DRAM-4 and 5 have a similar function in those cellular processes. In contrast to the most studied DRAM 1 (Crichton et al., Cell 2006), but like DRAM-3 (Mrschik et al., CDD 2015) expression of DRAM-4 and 5 is not induced by tumour suppressor p53. Elevated levels of DRAM-4 and 5 mRNA can be however, detected in response to starvation (EBSS). In addition, overexpression of full-length DRAM-5, but not DRAM-4, showed enhanced autophagic flux in basal and starvation-induced autophagy. Although the disruption of DRAM-5 did not show an altered autophagic flux, DRAM-4-deleted cells displayed higher basal autophagy. These effects might be explained by increased levels of DRAM-5 in those cells, since introducing the double knock-down of DRAM-4 and 5A reverses elevated basal autophagy levels. Interestingly and in contrast, DRAM-4 deletion also promotes survival in glucose or serum starvation conditions, with knock-down of DRAM-5B, but not DRAM-5A reversing this effect. Overall these studies provide new insights into the interconnected relationship between DRAM-4 and DRAM-5 in the regulation of autophagy, cell death and survival.

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 POSTER NUMBER: 2012

Regulation of p62-directed selective autophagy by TAK1
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The protein p62/Sequestosome 1 is a selective autophagy receptor that is also involved in a wide variety of autophagy-independent cellular processes. The mechanisms underlying how p62 coordinates these disparate activities are still unclear, although a number of posttranslational modifications that enhance the ability of p62 to act in autophagy have been identified. In this work, we demonstrate that TAK1, a serine/threonine kinase involved in canonical NF- κ B signaling, reduces the autophagic degradation of p62 and its poly-ubiquitylated protein targets.

Whereas TAK1 knockdown enhances the autophagy-mediated degradation of p62, it does not affect the autophagic targeting of the p62 homologue NBR1. Mechanistically, TAK1 and its binding partner TAB2 interact with p62 in a manner requiring K63-linked poly-ubiquitin chains. TAK1 expression promotes p62 phosphorylation at multiple sites, reduces the abundance of LC3B in p62 protein complexes, and reduces the localization of p62 to autophagosomes. Thus, TAK1 negatively regulates p62's function in selective autophagy and allows for the accumulation of both p62 and its substrates in cells. Additionally, TAK1 promotes the formation of cytoplasmic bodies containing p62, TAK1, and TAB2 that have liquid-like properties as demonstrated by FRAP experiments. Our data indicate that these TAK1-dependent p62 bodies constitute a separate pool of p62 that is disconnected from autophagy; raising questions as to their function. We propose that TAK1 acts as a switch that rebalances p62 action away from autophagy, allowing it to perform its autophagy-independent roles.

 POSTER NUMBER: 2013

Studies into the role of LC3/GABARAP delipidation by the four mammalian ATG4 isoforms

Upon induction of autophagy LC3/GABARAP proteins are conjugated to phosphatidyl-ethanolamine (PE) on the inner and outer membrane of autophagosomes to allow cargo selection and autophagosome formation. LC3/GABARAPs undergo two processing steps, the proteolytic cleavage of pro-LC3 and the de-lipidation of LC3-PE from autophagosomes, both executed by cysteine proteases of the ATG4 family. The role of de-lipidation in autophagosome-lysosome fusion has not been resolved to date, although recent evidence suggests that lipidated LC3 enables fusion by linking the fusion machinery to the autophagosome membrane. Here, we present new insights into the regulation of de-lipidation by the ATG4 family of proteins.

First, we hypothesized that ATG4B activity may be regulated by post-translational modifications. Specifically, we were intrigued by the fact that pro-LC3/GABARAP processing is fast and constitutive, while de-lipidation is slow and possibly regulated. We identified that ULK1 can bind to and phosphorylate ATG4B, leading to phosphorylation of Serine 316 in proximity to the active substrate recognition site. Phosphorylation at this residue results in inhibition of its catalytic activity *in vitro* and *in vivo*. On the other hand, phosphatase PP2A-PP2R3B can remove this inhibitory phosphorylation. We propose that the opposing activities of ULK1-mediated phosphorylation and PP2A-mediated de-phosphorylation provide a phospho-switch that regulates the cellular activity of ATG4B to control LC3 processing and de-lipidation.

Next, we investigated the role of the four ATG4 isoforms (ATG4A-D) with regards to the de-lipidation reaction. We identified that human HeLa cells lacking ATG4B exhibit a severe but incomplete defect in LC3/GABARAP processing and autophagy. By further genetic depletion of ATG4 isoforms we uncover that ATG4A, ATG4C and ATG4D all contribute to residual proteolytic activity, which is sufficient to enable lipidation of GABARAPL1 on autophagosomes. Intriguingly, we demonstrate that in the absence of all four ATG4 proteins, degradation of the cargo receptor p62/SQSTM1 can occur, indicating that de-lipidation of LC3/GABARAPs by ATG4 proteins is not essential for this process.

Overall, our results suggest that members of the ATG4 family of proteins can be phosphorylated by ULK1/2 to suppress the de-lipidation activity, and furthermore demonstrate that autophagosomes are capable of lysosome fusion and cargo degradation in the absence of ATG4 proteins.

POSTER NUMBER: 2015

Hypothalamic Mitochondrial Protein A Regulates Autophagy via AMP-Activated Protein Kinase

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Mito-A located in the mitochondrial outer membrane has emerged as a candidate regulator for cellular bioenergetics. However, the mediators and pathways involved are largely unknown. Autophagy, a self-degradative process of cellular components, contributes to energy production. Herein, we report Mito-A as a new regulator of energy metabolism via autophagy mediated by AMP-activated protein kinase (AMPK) in the adult mouse hypothalamic cell line. Pharmacological treatment of Mito-A antagonist or a knockdown of Mito-A induced autophagy in an AMPK-dependent manner. Moreover, ATP production was increased by the knockdown of Mito-A. A concomitant knockdown of ATG5 and Mito-A abolished the elevated level of ATP produced by the knockdown of Mito-A. Overall, our data unveil a novel regulatory pathway induced by hypothalamic Mito-A, changing cellular energy metabolism.

POSTER NUMBER: 2014

MIR144* inhibits antimicrobial responses against *Mycobacterium tuberculosis* in human monocytes/macrophages by targeting the autophagy protein DRAM2

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MicroRNAs (miRNAs) can regulate posttranscriptionally target gene expression. Tuberculosis (TB) is an infectious

disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb). However, the precise function of miRNAs is unclear in human TB. To evaluate the miRNA expression profiles of peripheral blood mononuclear cells (PBMCs) from patients with pulmonary TB, we analyzed 2 miRNA microarray datasets. Fourteen miRNAs in GSE 29190 were upregulated in active pulmonary TB patients compared with HCs. In addition, 180 miRNAs in GSE34608 showed increased expression levels in TB patients compared with healthy controls (HCs). The heatmap revealed that *MIR144** showed the greatest magnitude of upregulation among the 10 miRNAs. We then compared the expression of *MIR144** in PBMCs from active pulmonary TB patients and HCs. The expression levels of *MIR144** were significantly higher in PBMCs from active pulmonary TB patients than in HCs. In addition, *MIR144** expression was upregulated in samples collected from disease sites in pulmonary and extrapulmonary TB patients. We further found that *MIR144** expression was upregulated in human monocyte-derived macrophages (MDMs) after infection with Mtb in a multiplicity of infection (MOI)-dependent manner. These data indicate that *MIR144** expression is robustly increased in human MDMs following Mtb infection, and that *MIR144** levels are upregulated in PBMCs/tissues from TB patients compared with HCs.

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POSTER NUMBER: 1060

The effects of treatment modalities for refractory schizophrenia on AMPK-related autophagy system in the rat frontal cortex

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Clozapine is a gold standard for the management of treatment-resistant schizophrenia (TRS), however, it fails to elicit a response in 40-70% of TRS patients. Combined electroconvulsive therapy (ECT) and clozapine treatment has been applied and results in better treatment outcomes in patients with clozapine-resistant schizophrenia¹. According to our previous report⁴, clozapine induces autophagy via the AMPK/ULK1/Beclin1 signaling pathway in the rat frontal cortex^{2,3}, and electroconvulsive seizure (ECS) affects signaling pathways which interact with AMPK. We found that repeated ECS treatments for 10 days (E10X) increased p-Thr172-AMPK α immunoreactivity in the neurons of rat frontal cortex, including prefrontal, orbital, insular and cingulate cortices. E10X treatment also increased phosphorylation of upstream effectors of AMPK, such as LKB1, CaMKK, and TAK1, and of its substrates, ACC, HMGR, GABA_BR2, and ULK1. LC3-II and ATG5-ATG12 conjugate immunoreactivity was also increased after E10X, indicating activation of autophagy. An intracerebroventricular (i.c.v) injection of the AMPK inhibitor attenuated the ECS-induced increase in ULK1 phosphorylation, as well as the protein levels of LC3-II and the Atg5-Atg12 conjugate. The actual increase in the number of autophagosomes after the E10X treatment was also demonstrated by transmission electron microscopy. Taken together, our results show that repeated ECS treatments as well as clozapine activate autophagy through the AMPK signaling pathway *in vivo*, which could be one of the mechanisms for the synergistic effects of ECT and clozapine in the treatment of the patients with refractory schizophrenia.

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POSTER NUMBER: 2016

The intracellular Ca²⁺ channel TRPML3 is a PI(3)P effector that regulates early autophagosome biogenesis

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Autophagy is an intracellular degradation pathway that delivers cytoplasmic contents to the lysosome, involving many fusion and fission events which require Ca²⁺. Although TRPML3 is suggested to regulate autophagy as an autophagosomal Ca²⁺ channel, it is still not clear whether TRPML3 directly provides Ca²⁺ for the process. To image a TRPML3-mediated Ca²⁺ release in specific subcellular compartments, we used a GCaMP6-fusion protein, a genetically encoded calcium indicator attached to C-terminus of TRPML3. TRPML3-GCaMP6 hardly overlapped with LC3 but was mainly localized in ATG5-positive phagophores, indicating that TRPML3 supplies Ca²⁺ for the early autophagosome biogenesis. Moreover, nutrient starvation activated TRPML3 to release more Ca²⁺, suggesting that

TRPML3 are regulated in the context of autophagy. Indeed, lipid binding assay revealed that both N-terminus and 1st extracytosolic loop of TRPML3 interact with phosphatidylinositol-3-phosphate (PI3P), a key determinant of autophagosome formation. Confocal imaging and electrophysiological experiments showed that TRPML3 is directly activated by PI3P, resulting in increased autophagy. Inhibition of TRPML3 suppressed autophagy even in the presence of excess PI3P, whereas activation of TRPML3 rescued autophagy suppression. Part of the N-terminal and 1st extracytosolic loop sequences of TRPML3 was predicted as phox homology (PX) domain and RxL motif, respectively, which bind primarily to PI3P. Charge removal mutations in the regions disrupted binding to PI3P and abolished TRPML3 activation by PI3P and subsequent increase of autophagy. Taken together, these results suggest that TRPML3 is a key regulator of the autophagy process as a downstream effector of PI3P, providing Ca^{2+} that mediates early steps of autophagosome formation.

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POSTER NUMBER: 2017

The dynamic function of autophagy in the oleaginous microalga *Lobosphaera incisa*

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Microalgae are an extremely diverse group of unicellular, predominantly aquatic organisms, with the immense biotechnological potential and ecological significance. It has been recently demonstrated that nutrient stresses and other environmental cues induce or promote autophagy these organisms. Studies performed in model species indicated the importance of autophagy in lipid droplet biogenesis and degradation. We study the role of autophagy in the green non-model oleaginous microalga *Lobosphaera incisa*. This photosynthetic specie is distinguished by the unique capacity to accumulate triacylglycerols that are highly enriched in omega-6 LC-PUFA arachidonic acid, in the cytoplasmic lipid droplets under nitrogen starvation. We surmised that autophagy plays a crucial role in maintaining cell homeostasis and survival of *L. incisa* under stressful conditions.

Our results indicated that constitutive basal autophagy supports cell homeostasis of *L. incisa* under different growth stages and elevated under nitrogen or phosphate deprivation in accord with the lipid accumulation patterns. The dynamic formation and degradation of lipid droplets under conditions of different nutrient availability is associated with active functioning of cellular recycling systems as was evident by the expression patterns of *ATG8* gene and protein. Moreover, induction of autophagy by rapamycin increased the number of lipid droplets during nutrient-replete growth. Ultrastructural and microscopic analysis demonstrated that rapamycin administration enhanced the formation of acidic vacuoles and allowed visualization of diverse cytoplasmic cargo.

To monitor autophagy using a genetic approach, we created transgenic lines of *L. incisa* expressing *ATG8* fused with GFP under control of the native *LiATG8* promotor. The detection of GFP signals in cytoplasm indicated the active basal autophagy in the nutrient replete cells. Overexpression of *ATG8* delayed the chloroplast dismantling under nitrogen deprivation and promoted cell viability under ROS stress induced by methyl Viologen or H_2O_2 . Collectively, our data suggest the essential role of autophagy at the different growth stages in this organism.

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POSTER NUMBER: 2018

Stabilized Acinus manages cellular stress by elevating basal, starvation-independent autophagy

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Autophagy is a tightly regulated cellular process that supports survival during cellular stress. We have identified Acinus (Acn) as a key integrator of several cellular stress responses. Phosphorylation of its conserved serine-437 residue by Cdk5/p35 elevates basal levels of autophagy. Physiological relevance for this modification was confirmed by a phospho-mimetic mutation: Acn^{S437D} was stabilized and enhanced basal, starvation-independent autophagy thereby extending life span (1). Cdk5/p35-dependent phosphorylation of S437-Acn was elevated by expression of

aggregation-prone polyQ-containing proteins and the resulting elevated basal autophagy efficiently reduced polyQ accumulation. By contrast, loss of Cdk5/p35 activity compromised basal autophagy and promoted polyQ accumulation. Acn-S437 phosphorylation is highly dynamic and a genetic screen identified a metal-dependent protein phosphatase (CG6036) necessary for Acn-S437 dephosphorylation. Loss of CG6036 enhanced pS437-Acn levels and basal autophagy. Toxicological stress, such as cadmium poisoning, deactivate this family of phosphatases resulting in elevated levels of pS437-Acn and basal autophagy thus serving as a cytoprotective mechanism. As Acn is a subunit of the nuclear splicing-related ASAP complex, we considered alternative splicing as a possible mechanism for Acn to regulate autophagy. Acn^{ASAP}, a mutant that interrupts the binding interface with Sap18 and RNPS1, the two remaining ASAP subunits, disrupts Acn function in alternative splicing, as predicted. However, the Acn^{ASAP} protein is primarily cytosolic and enhanced basal autophagy, thus functionally separating Acn's function in splicing and autophagy. Further support for a splicing-independent, non-nuclear role of Acn came from expression of myristoylated Acn that also displayed enhanced autophagy. Taken together, our data indicate that in response to several cellular stressors phosphorylated Acn shuttles out of the nucleus and promotes basal autophagy to manage stress and maintain homeostasis.

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POSTER NUMBER: 2019

Autophagy in Aging Rat Intervertebral Discs and Other Body Tissues

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Introduction: Intervertebral discs (IVDs) are cartilaginous joints of the spine. IVDs consist of the outer annulus fibrosus (AF) surrounding the central nucleus pulposus (NP) tissue. The avascular, hypoxic, and nutrient deprived environment of IVD cells is different from most other body tissues. NP cells could be up to 8 mm away from the capillaries supplying its nutrients. How NP cells meet the energy demands to survive in a low nutrient environment is poorly understood. Autophagy is a cellular survival mechanism which is upregulated during nutrient deprivation. *We hypothesize autophagy is essential for NP cell survival/function and declines with age, which may contribute to age-related IVD degeneration.* **Methods:** We measured autophagy markers LC3-II/I and p62/SQSTM1 protein in NP cells and different body tissues by western blotting. **Cell culture:** Cells isolated from NP tissue of 6-month-old rats (n=3) were supplemented with low nutrient media (glucose free DMEM with 1% FBS, 1mM glucose) for 48 hours. Control cells were given α -MEM (5.5 mM glucose) with 10% FBS. **Tissue assessment:** Proteins were extracted from NP and AF tissues, kidney, muscle, and vertebral bone of 5 six-month-old rats. **Aging assessment:** Proteins were extracted from NP and AF tissues of 4 six-month old and 4 two-year old rats. **Results:** **Cell culture:** NP cells in low nutrient media, which mimics in vivo conditions, have significantly higher LC3-II/I protein ratio compared to control cells (p=0.01) but no change in p62/SQSTM1 expression. **Tissue assessment:** NP tissue has the highest basal level of LC3-II/I protein ratio in rats compared to AF tissue, kidney, muscle, and bone. **Aging assessment:** LC3-II/I protein ratio in disc tissue does not change with age. **Discussion:** Exposure to low nutrients significantly increased LC3-II/I protein ratio in NP cells *in vitro*. Aging assessment suggests disc autophagy may not decline with age. NP exhibited the highest level of LC3-II/I protein ratio compared to AF, kidney, vertebrae, and paraspinal muscle, suggesting robust basal autophagy level in NP tissue. On-going studies are being performed to demonstrate that autophagic flux is important for NP cell survival and function.

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POSTER NUMBER: 2020

Syntaxin 17 is a TBK1 substrate and phosphorylated syntaxin 17 plays a role in autophagy initiation

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The protein kinase TBK1 has been implicated in autophagy, with work hitherto primarily focused on interactions between TBK1 and selective autophagy receptors whereas additional studies have suggested a more generalized role in autophagosomal maturation or in trafficking events associated with ATG9. In this work, we show that TBK1 in addition to the previously reported functions, interacts with and modulates another autophagy factor, syntaxin 17 (Stx17). The interaction of Stx17 and TBK1 is reflected in phosphorylation of Stx17 by TBK1, which determines its intracellular localization. Surprisingly, these interactions and phosphorylation of Stx17 by TBK1 occur at the earliest stages of autophagy, i.e. at its initiation. We found that phosphorylation of Stx17 is important for formation of Atg13 and FIP200 puncta in response to starvation and that Stx17 knockout inhibits autophagy initiation. We show that phosphorylated Stx17 and parts of the initiation complex, localized to the Golgi in the resting state, respond to induction of autophagy by translocating from the Golgi to participate in the formation of mammalian pre-autophagosomal structure during autophagy initiation.

POSTER NUMBER: 2056

Title: Roles of actin cytoskeletal dynamics on cyclic Compression-induced autophagy in 3D collagen encapsulated human mesenchymal stem cells

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Hypothesis

Cells can perceive and propagate external forces to their interior via membrane mechanosensors and actin reorganization, then promoting the biogenesis of physiological structures consisted of vesicular cargoes. Of these, a specialized vesicle called autophagosome, generated by a lysosomal degrading process known as autophagy involving in energy and nutrient recycling upon the mechanical loading. Dysfunction of autophagy impairs the cell adaptation and survival, eventually leading to cell death and diseases. Recent studies report the mechanical forces can induce autophagy and enhance their survival in 2D cell models but there is little evident reported on 3D models. In this regard, this study hypothesizes that the dynamic compression loading can induce the autophagy mediated via a change in actin dynamics in human mesenchymal stem cells entrapped in type I collagen (3D MSCs).

Methods

To examine this hypothesis, we exerted a constant 10% strain of dynamic compression loading on 3D hMSCs with 5e5 cells/ml cell density at different duration (2hrs, 5hrs and 9hrs) and harvested them immediately after loading for subsequent detection assays including quantitation of the candidate markers for autophagy and actin remodeling and their distribution within cells by qPCR analysis and immunofluorescent (IF) staining respectively. We also included 2D monolayer and 3D hMSCs without compression for normalization and comparisons.

Results and conclusions

The qPCR analysis reveals differential expressions of autophagic (BL1 and LC3) and actin related markers (ARP2) at different duration. For autophagic markers, there are no apparent change until 5+0 and then reach a maximum at 9+0 for BL1, and however, it prominently moves upward from 2+0 and 5+0 and get to the highest LC3 expression at 9+0 for LC3. For ARP2, it drops to a minimum at 5+0 and then recovers to the normal at 9+0. In protein analysis, it consistently exhibits increased LC3II/LC3I ratio but reduced ARP2 expression at 9+0 demonstrating a divergent expression between LC3II/I and ARP2. Taken together, it suggests actin related proteins as key regulators, presumably enhancing the compression-induced autophagy despite further studies on the downstream effectors along the processes of autophagy and actin cytoskeletal dynamics will be required to make a better understanding of their actual interactions on the cell metabolism.

POSTER NUMBER: 2021

Give me a SINE: How Selective Inhibitors of Nuclear Export Modulate Autophagy and Aging

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The partitioning of proteins between the nucleus and cytoplasm is modulated by karyopherins that carry specific proteins in and out of the nucleus. We became interested in the role of karyopherins while investigating the regulation of the nuclear localization of the autophagy-regulating transcription factor EB (TFEB). Our laboratory has been investigating the nematode *C. elegans* ortholog of TFEB, called HLH-30, which modulates autophagy, lysosomal biogenesis and lifespan in a variety of long-lived mutants. Indeed, one of the major features of long-lived animals is the enrichment of specific transcription factors in the nucleus.

To find new genetic modifiers of HLH-30/TFEB, we screened for genes that, when silenced with RNAi, would lead to the enrichment of HLH-30 in the nucleus. The top candidate from our unbiased genome-wide RNAi screen in *C. elegans* was the nuclear export protein XPO-1 (XPO1, CRM1 or exportin 1 in mammals). Silencing *xpo-1* led to nuclear enrichment of HLH-30 in all RNAi-treated animals. Further characterization in nematodes led to the discovery that XPO-1 modulates HLH-30 function, autophagy and lifespan. Specifically, key transcriptional targets of HLH-30/TFEB were enhanced, which conferred increases in autophagosome and autolysosome formation.

As autophagy induction is a longevity-associated process ⁷, we investigated the effect of reducing XPO-1 on aging and found that it extended lifespan significantly in nematodes and in a manner similar to established longevity models. Indeed, we found that long-lived animals have reduced expression of *xpo-1*, suggesting that reduced nuclear export may improve the ability of these animals to maintain a longevity-associated nuclear protein profile. We anticipated that inhibiting nuclear export pharmacologically could possibly mimic the effect seen using *xpo-1* RNAi. We found that Selective Inhibitors of Nuclear Export (SINE) had beneficial effects on the lifespan of nematodes and ALS-afflicted flies, recapitulating our experiments with *xpo-1* RNAi. In HeLa cells, SINE compounds enhanced autophagy and lysosomal biogenesis in a TFEB-dependent manner demonstrating a conserved mechanism by which HLH-30/TFEB activity is modulated by nuclear export. In contrast to the mTOR inhibitor Torin1, SINE did not reduce mTOR activity, indicating that XPO1 inhibition is a viable avenue for stimulating autophagy and prevent neurodegeneration without affecting mTOR.

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POSTER NUMBER: 2022

Deciphering the roles of mitochondria-anchored E3 ligases

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Mitochondria are double membrane organelles important for metabolic function in the cell. Its dynamic nature, illustrated by constant fission and fusion mechanisms is essential to accomplish cellular processes like energy production[1]. Dysfunction in mitochondrial function was shown to be the cause of several diseases, including neurodegeneration[2, 3]. One particular aspect drawing a lot of attention is the specific degradation mechanism of damaged mitochondria by PINK1 and Parkin[4]. Because damaged mitochondria can be harmful for the cell, a cascade of cellular processes involving the stabilization of the kinase PINK1, the recruitment of the E3 ligase Parkin and ubiquitin tagging of outer mitochondrial membrane proteins is rapidly mobilized to isolate the damaged mitochondria and degrade it via a process called mitophagy[5]. Recent studies highlighted the presence of E3 ligases embedded in the outer mitochondrial membrane or targeted to the mitochondria[6]. Loss of iron was shown to trigger a PINK1/Parkin-independent mitophagy[7]. We here propose to investigate the molecular mechanisms of PINK1/Parkin-independent mitophagy triggered by iron chelators and the putative role of the mitochondria-anchored E3 ligases in this pathway. Using quantitative proteomics we could identify the specific ubiquitin substrates of mitochondria-anchored E3 ligases and identify their role in mitochondrial homeostasis.

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POSTER NUMBER: 2023

Deacetylation of CHK2 by SIRT1 modulates cellular homeostasis

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Growing evidence indicates that metabolic signaling pathways are interconnected to DNA damage response (DDR). However, factors that link metabolism to DDR remain incompletely understood. SIRT1, a NAD⁺-dependent deacetylase that regulates metabolism as well as aging, has been shown to protect cells from DDR. Here we demonstrate that SIRT1 protects cells from oxidative stress-dependent DDR by binding and deacetylating Checkpoint Kinase 2 (CHK2). We first showed that essential proteins in DDR were hyper-acetylated in Sirt1-deficient cells and that among them the level of acetylated CHK2 was highly increased. We found that Sirt1 formed molecular complexes with BRCA1/BRCA2-associated helicase 1 (BACH1), H2AX, Tumor suppressor p53-binding protein 1 (53BP1) and CHK2, which are key factors of DDR. We then demonstrated that CHK2 was normally inhibited by SIRT1 via deacetylation but dissociated with SIRT1 under oxidative stress conditions. This led to acetylation and activation of CHK2, which increased cell death under oxidative stress conditions. Our data also indicated that SIRT1 deacetylated K235 and K249 residues of CHK2, whose acetylation increased cell death in response to oxidative stress. Thus, SIRT1, a metabolic sensor, protects cells from oxidative stress-dependent DDR by deacetylation of CHK2. Our finding suggests a crucial function of SIRT1 that inhibits CHK2 as a potential therapeutic target for cancer treatment.

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POSTER NUMBER: 2024

On the existence of chaperone-mediated autophagy in fish

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Chaperone-Mediated Autophagy (CMA) is a major pathway of lysosomal proteolysis recognized as a key player in the control of numerous cellular functions, and whose defects have been associated to several human pathologies, including neurodegenerative diseases, cancers and immune disorders. To date, this cellular function was presumed to be restricted to mammals and birds, due to the absence of an identifiable LAMP2A, a limiting and essential protein for CMA, in other species. However, we recently identified the existence of expressed sequences displaying high homology with the mammalian LAMP2A in several fish species, challenging that view and suggesting that CMA appeared much earlier during evolution than initially thought. In the present study, we first present new evidences about the evolutionary history of the gene LAMP2 in vertebrates. We demonstrate that LAMP2 appeared after the second whole genome duplication that occurred at the root of the vertebrate lineage approximately 500 million years ago. By using a fluorescent reporter previously used to track CMA in mammalian cells, we then reveal the existence of a CMA-like pathway in a fibroblast cell line of the fish medaka (*Oryzias latipes*). We show that this CMA reporter localizes, upon long term starvation, to lysosome and late endosomes in a Lamp2a-dependent manner. Finally, to address the physiological role of Lamp2a in fish, we generated medaka knockout for the splice variant *lamp2a* and found severe alterations in hepatic carbohydrate and fat metabolisms upon fasting, as previously demonstrated in mice deficient for CMA in the liver. Collectively, these data provide the first evidence of a CMA-like process in fish and bring new perspectives on the possible use of complementary genetic models, such as zebrafish or medaka, for studying CMA function.

 POSTER NUMBER: 2057

The ULK1-FBXW5-SEC23B nexus controls autophagy

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In response to nutrient deprivation, the cell needs to mobilize an extensive amount of membrane to form and grow the autophagosome, allowing the progression of autophagy. By providing membranes and a source for LC3 lipidation, COPII (Coat Protein Complex II) localizes to the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) and promotes autophagosome biogenesis. However, the molecular mechanisms that, in response to starvation, divert COPII from the secretory pathway to the autophagic pathway are largely unknown. Here, we show that the F-box protein FBXW5 targets SEC23B, a component of COPII, for proteasomal degradation and that this event limits the autophagic flux in the presence of nutrients. In response to starvation, ULK1 phosphorylates SEC23B on Serine 186, preventing the interaction of SEC23B with FBXW5 and, therefore, inhibiting its degradation. Phosphorylated and stabilized SEC23B associates with SEC24A and SEC24B, but not SEC24C and SEC24D, and they re-localize to the ERGIC, promoting autophagic flux. Induction of autophagy and localization of both SEC23B and SEC24B to the ERGIC in response to nutrient deprivation are significantly reduced in SEC23B(S186A) knock-in cells. We propose that, in the presence of nutrients, FBXW5 limits COPII-mediated autophagosome biogenesis. Inhibition of this event by ULK1 ensures efficient execution of the autophagic cascade in response to nutrient starvation.

 POSTER NUMBER: 2058

Cross-Talk between Tlr9 and Beclin 1 regulates exercise-induced muscle AMPK activation

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The activation of adenosine monophosphate-activated protein kinase (AMPK) in skeletal muscle plays a key role in coordinating systemic metabolic responses to exercise. Autophagy, a lysosomal degradation pathway that maintains cellular homeostasis, is upregulated during exercise, and a core autophagy protein, Beclin 1, and its dissociation from BCL2 is required for skeletal muscle AMPK activation. In the current study, we examined the role of the innate immune sensing molecule, toll-like-receptor 9 (Tlr9), and its interaction with Beclin 1 in exercise-induced muscle AMPK activation. We found that mice lacking Tlr9 are deficient in exercise-induced AMPK activation and Glut4 plasma membrane localization, but not autophagy, in skeletal muscle and they also display reduced blood glucose clearance and exercise endurance. Tlr9 binds Beclin 1, and this interaction is increased by energy stress – glucose starvation *in vitro* and endurance exercise *in vivo*. Of the two Beclin 1-containing phosphatidylinositol 3-kinase complexes, Tlr9 regulates the assembly of the endolysosomal Beclin 1/UVRAG complex in skeletal muscle during exercise. Knockout of Beclin 1 or UVRAG inhibits glucose starvation-induced AMPK activation *in vitro*. Moreover, a knock-in mutation in BCL2 (previously shown to block disruption of BCL2/Beclin 1 binding and exercise-induced AMPK activation) inhibits the exercise-induced increase in Tlr9/Beclin 1 interaction. These findings reveal a heretofore undescribed role of the innate immune sensing machinery in muscle AMPK activation and glucose metabolism during exercise, as well as unexpected cross-talk between this machinery and autophagy proteins.

 POSTER NUMBER: 2025

Sustained Activation of Autophagy Suppresses Adipocyte Development via a Lipolysis-dependent Mechanism

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Autophagy is implicated in adipose tissue development and lipid metabolism. However, the mechanisms underlying the role of autophagy in lipid droplet (LD) expansion remain poorly defined. Using adipose-specific Raptor knockout (KO), ATG7 KO and ATG7/raptor double KO mice and the relative primary adipocytes, here we show that inhibiting mammalian target of rapamycin complex 1 (mTORC1) led to autophagic sequestration of LD and elevation of basal and isoproterenol-induced lipolysis *in vivo* and in primary adipocytes. Despite normal differentiation at early phase, progressive shrinkage and degradation of cellular LDs occurred in the terminal differentiation accompanied with downregulation of adipogenic markers PPAR γ , Plin1 and adiponectin of raptor KO adipocyte, which was rescued by inhibiting lipolysis or lysosome. In contrast, inactivating autophagy by depletion of ATG7 suppressed adrenoceptor-induced lipolysis and protected adipocyte against rapamycin treatment-induced lipolysis and LD degradation *in vitro*. Moreover, ATG7 deficiency decreased lipolysis, restored impaired adipocyte development and reversed fat loss in Raptor deficient mice *in vivo*. Ultimately, ATG7/Raptor double KO mice displayed enhanced thermogenic gene expression in BAT and energy expenditure compared to Raptor KO mice. Collectively, our study demonstrates that relatively lower level of autophagy favors adipocyte maturation by controlling lipophagy and lipolysis during adipogenesis.

 POSTER NUMBER: 2026

AMPK promotes xenophagy through 'priming' of autophagic kinases upon detection of bacterial outer membrane vesicles
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The autophagy pathway is an essential component of the innate immune response, capable of rapidly targeting intracellular bacteria, which are subsequently degraded by lysosomal enzymes. Recent work has begun to elucidate the regulatory signalling for autophagy induction in response to pathogenic bacteria. However, the initial signalling regulating autophagy induction in response to the detection of pathogens remains largely unclear. Here we report that AMPK signalling, an important upstream activator of the autophagy pathway, is rapidly stimulated upon detection of pathogenic bacteria, prior to bacterial invasion. Bacteria recognition is achieved through detection of outer membrane vesicles (OMV). Additionally, we show that AMPK signalling relieves mTORC1-mediated repression of the autophagy pathway in response to *Salmonella* infection, positioning the cell for a rapid induction of autophagy. Surprisingly, we found that the activation of AMPK and inhibition of mTORC1 in response to extracellular *Salmonella* are not accompanied by an induction of bulk autophagy. However, upon *Salmonella* invasion AMPK signalling is required for efficient and selective targeting of bacteria-containing vesicles by the autophagy pathway through activation of pro-autophagic kinase complexes. Collectively, these results demonstrate a key role for AMPK signalling in coordinating the rapid autophagic response prior to invasion of pathogenic bacteria.

 POSTER NUMBER: 2027

LC3-associated phagocytosis is a protective mechanism against inflammation during cirrhosis

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Sustained hepatic and systemic inflammation is a driving force for chronic liver disease progression to fibrosis and its end-stage cirrhosis. Convergent data demonstrate that the hepatic innate immune system, in particular monocyte/macrophages, governs initiation, perpetuation and maintenance of the inflammatory response, with major deleterious impact on liver injury and fibrosis. In patients with cirrhosis, sustained systemic inflammation drives the development of multiorgan failure and subsequent death. Therefore, there is growing interest in the identification of the mechanisms that control monocyte/macrophage phenotype to limit inflammation during chronic liver disease.

The involvement of autophagy in liver inflammation and fibrosis is only emerging. We have shown that mice lacking *ATG5* in myeloid cells are more susceptible to liver inflammation when exposed to chronic alcohol feeding or toxic injury, and show enhanced hepatocyte death and exacerbated fibrosis (Lodder J (2015), Denaes T (2016), Habib A (2018)). Here, we postulated that LC3-associated phagocytosis (LAP) might contribute to the regulation of inflammation and fibrosis. Combining studies in blood and liver samples from cirrhotic patients, and experiments in mice specifically deleted for LAP components in myeloid cells, we report that i) LAP, but not macroautophagy, is constitutively enhanced in blood and liver monocytes from cirrhotic patients, and is negatively correlated to the levels of inflammation markers in these patients; 2) genetic invalidation of Rubicon in myeloid cells (LAP-deficient mice) or pharmacological inhibition of LAP components enhance the inflammatory profile of blood and liver monocytes; iii) Myeloid-specific Rubicon-deficient mice are more prone to inflammation and liver fibrosis; iv) constitutive LAP activation in monocyte/macrophages is driven by FcγRIIA activation. These data shed light on a novel protective role for LAP against inflammation and fibrosis during chronic liver disease.

POSTER NUMBER: 2028

Harnessing Autophagy for the therapeutic benefit of cutaneous melanoma

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Melanoma, the most devastating of skin cancers continues to increase globally year on year. The introduction of targeted/ immunotherapies has undoubtedly revolutionized treatment options for patients with metastatic disease, however the efficacy of such therapeutic approach is harbored by a lack of credible biomarkers able to identify or stratify high risk patient subgroups. Moreover, in an era of personalized medicine an acute need for effective precision based therapeutics able to overcome deregulated cellular signaling in melanoma remains. Autophagy deregulation in melanoma is well acknowledged for which we show the semi quantitative immunohistochemical (IHC) expression of p62 in primary tumours mirrors the paradoxical role of autophagy in cancer, and specifically, is a prognostic biomarker for patients with AJCC stage II disease. Autophagy modulation in melanoma therapy is also a hot topic in cancer medicine and we additionally show its specific inhibition through the use of Vps34 inhibitors or the use of selective cannabinoid derivatives to promote cytotoxic autophagy as an effective strategy to drive melanoma cell death both *in vitro* and *in vivo*, for which p62 may also serve as a biomarker of response. Finally our studies of the IHC expression of pro-autophagy regulatory AMBRA1 in the epidermis overlying primary AJCC stage I melanomas and in vascular endothelial cells within surrounding blood vessels demonstrates loss of epidermal/endothelial expression results in compromised epidermal/endothelial integrity thereby facilitating tumour metastasis. In this context we show loss of peri-tumoural epidermal AMBRA1 in combination with loss of the IHC expression of the epidermal marker loricrin is a prognostic biomarker and a companion biomarker for novel TGFβ signaling targeted adjuvant therapy.

Collectively our data demonstrate how autophagy regulatory proteins can be translated as prognostic/predictive and companion biomarkers for melanoma together with novel therapeutic approaches through which autophagy modulation can be harnessed as an effective treatment strategy for metastatic disease.

 POSTER NUMBER: 2029

Autophagy and tissue repair: sunburn, vitamin D, and macrophages
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In human skin, vitamin D facilitates tissue repair in sunburns following ultraviolet (UV) radiation exposure. Recovery is associated with anti-inflammatory M2 macrophages that work to suppress inflammation and promote skin regeneration. Since vitamin D is known to enhance cellular autophagy in macrophages we sought to investigate whether autophagy is critical in vitamin D protection from UV-mediated injury. Using an experimental acute UV injury mouse model, we demonstrate that a single dose of vitamin D resolves tissue damage with sustained inhibition of inflammatory cytokines and enhanced autophagy in myeloid anti-inflammatory M2 macs. Increased expression of autophagy markers LC3 and increased degradation of p62 in skin tissue was observed with vitamin D intervention. Enhanced macrophage autophagy is observed using fluorescence microscopy for LC3 expression and detection of autophagolysosome formation by electron microscopy. In vivo pharmacological inhibition of autophagy with 3-MA results in increased UV-induced epidermal apoptosis with suppressed M2 macrophages populations in the skin. Furthermore, 3-MA treatment blocks the ability of vitamin D to downregulate the critical tissue destructive mediators, TNF- α and MMP9, in the skin. Selective deletion of autophagy in myeloid cells using the LysM-Cre-ATG7 (Atg7cKO) conditional knock-out mice blunts the protective effects of vitamin D and recapitulates the UV-induced inflammatory phenotype. Mechanistically, vitamin D initiates a signaling cascade characterized by activation of M2-autophagy regulators Klf4 (Kruppel-like factor 4), PPAR γ (peroxisome proliferator-activated receptor gamma) and Arg1 (arginase1). Lastly, examination by confocal microscopy of skin biopsies taken from human subjects exposed to UV in vivo confirm LC3 co-localization with cells expressing the human M2 macrophage marker CD163. This is increased in subjects who received a single high dose intervention with oral vitamin D vs. placebo. Taken together, the data suggests that recovery from sunburn is chiefly mediated by M2 macrophages to promote tissue repair and that vitamin D induction of autophagy may be a critical step in the differentiation of M2 macrophages in tissue injury.

 POSTER NUMBER: 1062

Potential for Synergistic Effect of Proprietary Autophagy Inducing Molecules in the Treatment of Tuberculosis
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Mycobacterium tuberculosis infects one third of the world's population, approximately two billion people, claiming more lives than many other pathogens due to drug resistant strains prevalent in both third world and advanced countries. Because of this, there is an urgent need for more efficacious and faster treatment regimens. Previous studies Mizushima, N., et al. (2008). "Autophagy fights disease through cellular self-digestion." Nature 451: 1069.

*Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-NA0003525. indicate that autophagy can stimulate the immune system to attack invading bacteria and thereby potentiate antibiotic activity. This study focused on four clinically approved small molecules (rapamycin, bromhexine, flubendazole, niclosamide) and several proprietary, autophagy stimulating compounds in combination with a standard tuberculosis (TB) antibiotic to identify a more robust, faster acting treatment. Confocal fluorescence imaging and a HeLa cell line expressing autophagy biomarkers (LC3 fused to GFP and RFP) was used to quantify autophagy stimulation at the single cell level (4 and 18 hrs.). Measurements included the intensity, area, and number of GFP, and RFP puncta present with autophagy maturation observed as a decrease in GFP puncta with a concurrent increase in RFP puncta. Antibiotic potentiation was determined using a standard TB antibiotic (isoniazid) in RAW 264.7 cells infected with *M. bovis*, a TB surrogate. While all the compounds modulated autophagy to some degree and in distinct ways,

niclosamide was the most effective of the approved compounds in stimulating autophagy while two proprietary compounds displayed high levels of autophagy stimulation at either 4 or 18 hours. In addition, niclosamide as well as several of the proprietary compounds potentiated isoniazid killing against *M. bovis* and some proprietary compounds showed significant antibiotic activity on their own, perhaps through autophagy stimulation. Finally, we assessed the mechanism of action for the proprietary compounds through the inhibition of Nf-kB dimerization through translocation of GFP-RelA into the nucleus.

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POSTER NUMBER: 2030

TAK1 switches p62 from an autophagy receptor to a signaling platform

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Effective maintenance of cytoplasmic quality requires the recognition and elimination of unnecessary or undesired cellular contents. Autophagy contributes to this process, with receptors such as p62/Sequestosome 1 conferring selectivity. Our understanding of how the selective autophagy machinery is regulated to discriminate between potential substrates is still incompletely developed. In this study, we have found that the kinase TAK1, a key component of immune signal transduction pathways, functions as a negative regulator of p62 action in autophagy. TAK1 dampens the autophagic degradation of both p62 and of known substrates of p62-directed autophagy and reduces p62 localization to autophagosomes. Instead, TAK1 promotes the coalescence of p62 into dynamic cytoplasmic specks which may play a role in other non-autophagy functions such as signaling. Indeed, TAK1 localizes to p62 specks and p62 speck formation is associated with the stabilization of TAK1 complex components; the assembly and activation of which require p62. In summary, TAK1 governs p62 action, switching it from an autophagy receptor to a platform for inflammatory signaling. This ability to disable selective autophagy receptors constitutes an additional level of precision in the control of cytoplasmic contents and may allow certain autophagic substrates to accumulate when needed for cellular functions.

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POSTER NUMBER: 2031

Phagolysosomal signaling regulates inflammasome positioning and silencing by autophagy in dendritic cells

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Phagosomes in dendritic cells (DCs) function as sensing and signaling organelles that link phagocytosis to the appropriate immune responses according to the encountered threat. Microorganisms captured by phagocytosis are first sensed through the detection of conserved features by pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) located at the cell surface and on phagosomes. Other PRRs localize to the cytosol, sense pathogen-derived products that escape phagosomes, and therefore signal to a "higher threat" level of immune response. Assembly of cytosolic PRRs within a multisubunit complex called the inflammasome escalates the inflammatory response. We previously showed that the endosomal adaptor protein AP-3 is required for optimal TLR signaling from phagosomes in DCs, regulates the positioning of assembled inflammasomes in the perinuclear area and limits autophagy in order to sustain inflammation and control bacterial infection. AP-3 mediates the sorting of transmembrane proteins (such as TLRs and nutrient transporters) to lysosomes and phagosomes. We thus propose that AP-3-mediated recruitment of nutrient transporters to phagolysosomes may allow the recognition of phagosomal degradation products and trigger the autophagy program by modulating the activity of the nutrient sensor and master cell growth regulator mammalian target of rapamycin (mTOR) at the phagosomal or lysosomal level. Our new data show that knock-down of the AP-3 cargo and peptide transporter SLC15A4 or the nucleoside transporter SLC29A3, which are associated with human inflammatory diseases, but not the fatty-acid transporter SLC27A4, leads to reduced inflammasome activity (measured by IL-18 secretion and pro-caspase-1 cleavage) after *Salmonella Typhimurium* infection or alum stimulation in DCs. This correlates with peripheral inflammasome assembly, decreased mTOR signaling and increased inflammasome sequestration by autophagosomes (assessed by immunofluorescence microscopy and immunoblotting). We propose that phagolysosomal nutrient sensing couples phagocytosis to inflammasome activation and silencing by autophagy through the modulation of mTOR signaling

pathways.

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POSTER NUMBER: 2033

Mechanisms of Selective Autophagy - From Phase Separation to Cargo Degradation

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Autophagy is an intracellular lysosomal bulk degradation pathway that ensures cellular homeostasis by the removal of damaged and dangerous material from the cytoplasm. This is achieved by the sequestration of the cytoplasmic cargo material within double membraned organelles called autophagosomes. The selective sequestration of only specific cargo material is mediated by cargo receptors that link the cargo to the nascent autophagosomal membrane. How cargo selection, membrane nucleation and growth are coupled is unclear. I will present our recent work on the human cargo receptors and the autophagy machinery derived from *in vitro* reconstitution systems and cell biology. In particular, I will discuss how the p62 cargo receptor and the autophagy machinery including FIP200 and ATG8 proteins act sequentially during cargo condensation, membrane nucleation and elongation to mediate the specific sequestration and subsequent degradation of cellular material.

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POSTER NUMBER: 2034

Autophagy deficiency in vascular smooth muscle cells affects passive and active aortic vessel compliance in young C57Bl6 mice

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An independent and early hallmark of cardiovascular disease such as hypertension and atherosclerosis is an increase in stiffness of the large arteries. Because the incidence of cardiovascular disease increases with age and aging is associated with a decline in vascular autophagy, we speculated that autophagy deficiency increases vascular stiffness and thus decreases arterial compliance. Vascular stiffness of aortic segments isolated from young (2 months old) mice with normal autophagy or an autophagy defect (*Atg7* deletion via Cre-loxP technology) selectively in vascular smooth muscle cells (VSMCs) was investigated using an in-house developed Rodent Oscillatory Tension Set-up to study Arterial Compliance (ROTSAC) at physiological pressures (70-150 mm Hg) and frequencies (10 Hz). In basal unstimulated conditions (5.9 mM K⁺) and at normal physiological pressures, the Peterson's modulus (Ep), which is a vessel diameter independent measure for arterial stiffness, was not significantly different in *Atg7*^{F/F} SM22-Cre mice as compared to *Atg7*^{+/+} SM22-Cre control mice. However, at increased distention pressures, Ep values were significantly higher in *Atg7*^{F/F} SM22-Cre mice. This difference in vascular stiffness was similar after the removal of extracellular Ca²⁺, indicating that passive aortic wall remodeling rather than differences in VSMC tone is responsible for the difference in stiffness, which is in line with histological data showing an increase in aortic medial wall thickness. Differences in vascular stiffness at higher pressures were enhanced by depolarizing aortic segments with high K⁺, suggesting that in these conditions active tonus of the segments also contributed to the different aortic stiffness. These findings were confirmed in a traditional isometric organ bath setup. Aortic segments of *Atg7*^{F/F} SM22-Cre mice showed elevated contractions and were more sensitive to depolarization (high K⁺). Overall, our data demonstrate that autophagy deficiency in VSMCs plays an important role in arterial wall compliance and suggest that pharmacological induction of autophagy might be a novel approach to treat vascular stiffness.

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POSTER NUMBER: 2059

Calcimycin-induced IL-12 production enhances autophagy and restrict intracellular mycobacterial growth

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Calcium ionophores like calcimycin is well known for their antibacterial activity but their antimycobacterial activity is still in its infancy. We reported earlier that calcimycin restrict the intracellular mycobacterial growth by enhancing autophagy, one of the prominent host-defence pathways in a Purinergic Receptor, P2RX7 dependent manner by modulating intracellular calcium-modulated ATP release. We next got interested in looking at the role of immune regulators in regulation of calcimycin-induced autophagy against intracellular mycobacteria in THP-1 macrophages. We found significant mRNA expression of Interleukin-12 (IL-12) in calcimycin treated macrophages than other pro-inflammatory cytokines like Tumour Necrosis Factor- α (TNF- α) and IL-18 through time kinetic experiment. Calcimycin treated macrophages also showed increased production of IL-12 with concomitant expression of IL-12 receptor at mRNA level. P2RX7 inhibitor

1-[N,O-bis(5-Isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) and siRNA based P2RX7 inhibition abrogated release of IL-12 upon calcimycin treatment by affecting Jun N-terminal kinase (JNK) - NF- κ B signalling pathway. Specific inhibitors against JNK and NF- κ B molecules showed decreased IL-12 production and less autophagy in calcimycin treated cells that lead to a significant intracellular mycobacterial growth. Experiment with IL-12 neutralizing antibody conclusively proved that IL-12 acts in an autocrine fashion to regulate autophagy-dependent intracellular mycobacterial growth in calcimycin-treated cells. Collectively, our study showed that calcimycin through P2RX7 binding modulates intracellular JNK-NF- κ B signaling pathway which in turn regulates IL-12 mediated autophagic pathway in an autocrine fashion that inhibit intracellular survival of mycobacteria in THP-1 macrophages. So, understanding the mechanism of autophagy by calcimycin may provide an attractive target for the control of mycobacterial infection, which will help in developing better therapeutic interventions against tuberculosis.

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POSTER NUMBER: 3056

Increased lifespan through altered Gcn4 / ATF-5 in *S. cerevisiae* and *C. elegans*.

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In a recent whole-genome screen for deletions that increase lifespan in *S. cerevisiae*, we identified increased Gcn4 signaling as a mediator of increased lifespan. Gcn4 is a nutrient-responsive transcription factor whose entire pathway is functionally conserved from yeast through humans. Accumulation of uncharged tRNAs has been shown to upregulated Gcn4 and its mammalian ortholog, ATF4. Here we demonstrate that chemical inhibitors of tRNA synthetases significantly extend lifespan in both yeast and the nematode *C. elegans* in a dose- and Gcn4-dependent manner, and that this is in part due to changes in autophagy.

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POSTER NUMBER: 2035

Defective lysosome homeostasis during autophagy causes skeletal muscle disease

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Skeletal muscle is heavily reliant upon the cytoprotective functions of autophagy and its inhibition causes disease in mice and humans. During autophagy lysosomes are heavily consumed, via their fusion with autophagosomes, before being replenished to steady state levels. *In vitro* cellular studies have shown that if lysosomes are depleted, autophagy becomes progressively suppressed. Two alternate pathways regulate lysosome repopulation during autophagy; TFEB/TFE3-dependent lysosome biogenesis and autophagic lysosome reformation (ALR). The microphthalmia-associated transcription factors (MiTF) TFEB/TFE3 are master regulators of the lysosomal system, controlling the expression of nearly all genes required for *de novo* lysosome formation. ALR recycles existing autophagic membranes to reform lysosomes. In skeletal muscle basal autophagy is high, and is further increased as an adaptive response to exercise or fasting. As such, we predicted that muscle would have a high demand for lysosome generation to maintain continued autophagic flux. Here we report for the first time that defective lysosome homeostasis in muscle causes marked inhibition of the autophagy pathway and severe muscle disease. We define a novel mechanism for regulating lysosome production during autophagy through the spatiotemporal regulation of key phosphoinositides. Our study further identifies how lysosomes are maintained in muscle *in vivo* and how failure of this process causes disease. This represents a new disease pathway in muscle which we predict will have broader implications for other disorders where autophagy is important including neurodegenerative conditions.

POSTER NUMBER: 2036

Role of exosome-secreted non-coding RNAs in autophagy regulation and neuronal and glial homeostasis -implications for psychiatric disorders

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Autophagy is a coordinated lysosomal process for the degradation and recycling of cellular components, organelles, and protein aggregates that has been heavily implicated in the pathophysiology of neurodegenerative disorders. However, recent data suggest that autophagy is an important regulator of normal neuronal and glial functions. Moreover, numerous autophagy-associated genes display reduced expression in postmortem brains of subjects with psychiatric disorders, while the majority of mood stabilizers and antipsychotics appear to activate autophagy. Furthermore, inducing autophagy in mouse models of schizophrenia and depression has been shown ameliorate neuronal and glial dysfunction and improve disease-related behavior. It is, therefore, apparent that in addition to its relevance for neuronal survival during neurodegeneration, autophagy, could also play an important role in regulating normal neuronal and glial homeostasis and function with implications for psychiatric disorders. Despite the above, the importance of autophagy in psychiatric disease pathogenesis and pathophysiology still remains underexplored. We have uncovered alterations in the expression of two non-coding RNAs in postmortem brains of subjects with schizophrenia (a glial produced inflammation-induced miRNA and a neuronal-enriched activity-dependent circular RNA), both of which have been previously shown to differentially regulate autophagy-related genes. Both non-coding RNAs (ncRNAs) appear to respond to either antipsychotic or mood stabilizer treatment and to be secreted via exosomes. Moreover, *in vivo* manipulation of their expression can affect neuronal gene expression and function. Ongoing experiments are aiming in determining the mechanisms of exosomal integration, secretion, and uptake of these brain ncRNAs with relevance to psychiatric disease and in examining their effects on neuronal and glial autophagy and cellular homeostasis.

POSTER NUMBER: 2037

Regulation of endosomal Microautophagy in Drosophila

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Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. As such, autophagy counteracts various human diseases, and its reduction leads to aging like phenotypes. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but

selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs.

Using a KFERQ-tagged fluorescent biosensor, we have identified an eMI-like pathway in the genetically easily tractable model organism *Drosophila melanogaster*. Upon starvation, this biosensor localizes to late endosomes/lysosomes in an Hsc70- and ESCRT machinery dependent manner. Currently, we are characterizing the physiological role of eMI in flies with a focus on what types of cellular stress and by what mechanism eMI is activated. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response, implying a selectivity of the process.

The DNA damage response is well studied and acts both as a sensor for damage and as effector to help the cell contain said damage and is thus critical to prevent cancer, neurodegeneration, and heart disease. Based on the initial observation that eMI is DNA damage inducible in flies, we are testing the hypothesis that this activation occurs through the ATM/ATR DNA damage response pathway. The ATM/ATR kinases are responsible for controlling DNA repair mechanisms, cell cycle arrest and apoptosis. Using genetic analyses, our initial results show that indeed ATM/ATR kinases can act as eMI modulators that likely control the recycling of proteins through multi-vesicular body formation in late endosomes.

POSTER NUMBER: 2038

Autophagy mediates human endometrial stromal cells differentiation

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The human endometrium, govern by plasma levels of ovarian steroid hormones, undergoes cyclic changes of proliferation, differentiation and tissue regeneration^{1,2}. The differentiation of the endometrium, named decidualization, is critical to ensure proper materno-fetal interactions and pregnancy success².

Macroautophagy (autophagy) is an intracellular degradation pathway that plays an important role during embryo development and cellular proliferation, differentiation and growth.

In the endometrium, the increase detection of the autophagy marker LC3BII by western blot correlates with the progression of the menstrual cycle, reaching its maximum towards the late secretory phase³. Besides this, autophagy has been study in relation to the onset of endometriosis, a benign fertility-related endometrial pathology in which endometrial tissue attaches and growths outside the endometrial cavity⁴. However, to date, no functional assay has been performed to connect autophagy with endometrial pathophysiology.

To define the role of autophagy during endometrial cells differentiation we evaluated changes in autophagic flux by flow cytometry in an *in vitro* model of decidualization using immortalized human endometrial stromal cells (T-HESC). Furthermore, we transfected T-HESC with specific siRNA against two ATG proteins, both components of the core machinery of autophagosomes formation, and we evaluated the level of expression of prolactin (PRL), a well-known marker of decidualization.

Our results show that autophagy flux is modulated during decidualization and that autophagy is in part responsible for the correct differentiation of endometrial cells, as knockdown experiments with ATG specific siRNA lead to changes in the mRNA and protein expression levels of PRL. Our next goal is to define the exact stimulus that activates autophagy in human endometrial stromal cells and to evaluate whether in these cells autophagy is a selective process.

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Mutating Specific Residues of Crucial Yeast Selective Autophagy Protein Atg11 Disrupts Interaction with Binding Partners

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Selective autophagy requires the coordinated assembly of dozens of proteins that assemble in a hierarchical order to form the pre-autophagosomal structure (PAS). Atg11, a protein crucial for selective autophagy in yeast, contains two coiled-coil domains, CC2 and CC3, which are the primary binding sites used to organize the autophagy initiation complex and recruit other autophagy proteins to the PAS. Here we use a yeast-2-hybrid system to investigate the effect of directed mutagenesis of selected residues within the Atg11 CC2 region on interaction with binding partners. We use a variety of mutation types to elucidate subtle differences between binding partners. Mutations in CC2 that induced a loss of interaction with those proteins believed to interact with the CC2 region (Atg1, Atg9 and Atg11 self-interaction) also disrupted the ability to bind Atg32, which has been shown not to require CC2 for its interaction, but instead bind to Atg11's C-terminal domain. This raises the possibility that these point mutations may be affecting protein folding and perhaps stability, which we are investigating by monitoring protein expression levels.

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Stepping up Validation of Autophagy Antibodies: Bio-Techne's Knockout Validation Initiatives

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Autophagy is a fundamental cellular catabolic process for the removal of damaged proteins and organelles to maintain cellular homeostasis. As such, autophagy plays critical roles in physiological and pathophysiological processes. Significantly, the autophagic molecular machinery and key autophagy substrates have been implicated in the etiology of various neurodegenerative diseases. A hallmark of neurodegeneration is the presence of key aggregated proteins, many of which are recycled through autophagy, and autophagy dysfunction has been linked to Parkinson's and Alzheimer's disease. However, the relationship between autophagy dysfunction and the onset or progression of neurodegeneration remains unresolved.

In an effort to provide worry-free and dependable reagents, Bio-Techne has adopted a series of validation standards (5-Pillar Validation) for its antibody portfolio, guaranteeing precise and reproducible detection. Among the current validation criteria, knockout validation provides a unique level of stringency. Therefore, by leveraging the strengths of CRISPR/Cas9 gene editing technology, Bio-Techne is advancing the validation of antibodies for key autophagy targets.

To leverage new therapeutic opportunities focused on autophagy manipulation, it is necessary to elucidate when and how autophagy dysfunction comes into play in the neurodegenerative process. Therefore, research tools must allow precise analysis of autophagy activity or "flux", and to monitor key proteins and genes controlling autophagy as well as critical targets involved in neurodegeneration.

Bio-Techne, through its various brands including R&D Systems, Novus Biologicals, Tocris, Protein Simple and Advanced Cell Diagnostics, provides cutting edge instruments and validated reagents to facilitate autophagy discovery.

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Autophagy is required for formation of surfactant-containing lamellar bodies in alveolar and swimbladder epithelial cells

Pulmonary surfactant, which is mostly composed of phospholipids, is essential for reducing surface tension at the air-liquid interface of the alveolar space to maintain lung volume. The surfactant is stored in the lamellar body, a lysosome-related organelle, in type II alveolar cells and secreted to the alveolar space. The mechanisms by which phospholipids are delivered to the lamellar body have not been fully understood. Here we found that autophagosomes frequently colocalized with lamellar bodies in type II alveolar cells in mice. Electron microscopic analysis occasionally demonstrated the presence of cytosolic materials within prolamellar bodies. Deletion of *Atg101* in mice caused a decrease in the amount of lipid contents and size of lamellar bodies in type II alveolar cells without affecting lipid synthesis, suggesting that autophagy is required for delivery of phospholipids to lamellar bodies. Type II alveolar cell-specific *FIP200* knockout mice showed cyanosis, gasping respiratory movements, and neonatal lethality. Zebrafish lacking upstream *autophagy-related* (*ATG*) genes (i.e. *FIP200*, *Atg101*, *Atg2a/b*, and *Vmp1*) also showed defective inflation of the swimbladder, an anatomical homologue of the lung. In these zebrafish, smaller lamellar bodies containing less lipids accumulated in swimbladder epithelial cells. By contrast, deletion of the downstream *ATG* gene *Atg5*, which is not absolutely essential for formation of the isolation membrane and autophagy, did not impair the functions of the lung and swimbladder in mice and zebrafish, respectively. These results suggest that autophagy is required for maturation of lamellar bodies in the lung and swimbladder.

POSTER NUMBER: 2041

Salt-inducible kinase 2 promotes autophagy in adipocytes

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Background: Autophagy is essential for adipocyte homeostasis and dysregulation of autophagy has been observed in obesity and type 2 diabetes. Salt-inducible kinase 2 (SIK2), a member of the AMPK-related kinase family, is downregulated in adipocytes from insulin resistant individuals and was previously demonstrated to regulate autophagy in cancer- and normal cell lines.

Aim: The aim of this study was thus to test the hypothesis that SIK2 regulates autophagy in adipocytes. If so, down-regulation of SIK2 in obesity could contribute to dysfunctional autophagy and the development of insulin resistance.

Methods: SIK2 expression was modulated by pharmacological inhibition and siRNA silencing in mature 3T3-L1 mouse adipocytes. Expression of the master transcription factor for autophagy, TFEB, was analysed by Western blot and qPCR. TFEB target genes were evaluated by qPCR. Autophagic flux was measured by Western blot as LC3-II turnover in the presence of the lysosomal inhibitor bafilomycin A1.

Results: We found that SIK2 inhibition and silencing resulted in reduced autophagic flux already after 1h of SIK2 inhibition. mRNA- and protein levels of TFEB were also decreased, although at time points *after* which autophagic flux was reduced. At later time points after SIK2 inhibition, nuclear TFEB levels and transcription of TFEB target genes were increased, perhaps as a compensation of the inhibition of autophagic flux.

Conclusion: Our data demonstrate that SIK2 promotes autophagy in mature adipocytes. We believe that this might be mediated via the regulation of TFEB expression, as well as through a distinct and more acute effect of SIK2, potentially directly on the autophagic machinery. These findings suggest that SIK2 down-regulation in obesity might contribute to autophagy dysregulation in adipocytes. Identifying the exact mechanism whereby SIK2 regulates autophagy represents a future challenge.

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Phosphorylated HSP27 to predict the role of Gemcitabine induced autophagy in osteosarcoma

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Metastatic osteosarcoma (OS) is lethal. Development of new therapies is imperative. Autophagy, a cell survival mechanism induced by stress, is associated with response/resistance to chemotherapy. We previously demonstrated aerosol gemcitabine (GCB) efficacy against OS lung metastasis and GCB to induce autophagy which was demonstrated *in vitro* by an increase in microtubule-associated light chain 3 two (LC3II) and Beclin 1 expression and a decrease in p62 in the LM7 and CCH-OS-D human metastatic OS cells. Inhibition of autophagy by hydroxychloroquine (HCQ) and shRNA targeting Beclin 1, enhanced LM7 cells sensitivity to GCB and decreased CCH-OS-D cells sensitivity to GCB, concluding that GCB-induced autophagy can contribute to either cell survival (LM7) or cell death (CCH-OS-D). What determines whether GCB-induced autophagy contributes to cell survival or death has been the focus of our studies. Using a kinase array, we demonstrated pAKT and pMTOR to be implicated in GCB-induced autophagy in both OS cell lines, however, phosphorylated heat shock protein 27 (pHSP27) was the only protein that was differentially expressed in both cell lines.

The aim of this study is to investigate if induction of pHSP27 predicts the role of GCB-induced autophagy in metastatic OS. HSP27 is a small heat shock protein associated with treatment resistance. We hypothesize that when pHSP27 is induced, blocking autophagy will lead to increased sensitivity to GCB.

We demonstrated that pHSP27 is significantly higher in GCB-treated LM7 cells as compared to CCH-OS-D cells. Inhibition of HSP27 by shRNA targeting HSP27 has no effect on autophagy induction in LM7 cells. Treatment of shHSP27LM7 with GCB resulted in an increase in LC3I/LC3II conversion and a decrease in p62. This was further confirmed *in vivo*. Transmitted electron microscopy of shHSP27 lung metastasis from mice treated with GCB showed an increase in the number of autophagosomes confirming induction of autophagy in the absence of HSP27.

Lastly, inhibition of autophagy in shHSP27LM7 cells using the combination GCB+HCQ resulted in increased viability as compared to the LM7 GpZ control cells confirming preliminarily our hypothesis that when pHSP27 is induced, blocking autophagy will lead to enhanced sensitivity.

LC3B phosphorylation regulates FYCO1 binding and directional transport of autophagosomes

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Macroautophagy relies on the intracellular transport of cargo-loaded autophagosomes (APs) to allow their fusion with lysosomes and cargo degradation. For this, the transport machinery of the cell promotes the anterograde (towards cell periphery) or retrograde (towards cell nucleus) transport of APs. Once mature, APs migrate mainly in a retrograde fashion to fuse with lysosomes in the perinuclear area. However, the molecular mechanisms that regulate the timing and direction of APs transport remain unclear. Our lab described a novel autophagy regulatory mechanism involving phosphorylation of the protein LC3B by the Hippo kinases STK3 and STK4 (Wilkinson et al., Mol Cell, 2015). Specifically, inhibition of LC3B phosphorylation causes a block in autophagy, which is accompanied by aberrant positioning of APs and lysosomes in the cell. To investigate the molecular basis for these findings, we employed proteomic analyses and identified that LC3B phosphorylation decreased the interaction of transport-related proteins to LC3B, including FYCO1. FYCO1 recruits the molecular motor kinesin, involved in anterograde transport. Accordingly, we hypothesized that LC3B phosphorylation may affect APs transport. Interestingly, the absence of LC3B phosphorylation was accompanied by an aberrantly high anterograde and a low retrograde transport of LC3B-positive vesicles. Moreover, starvation-induced perinuclear positioning of APs was prevented in the absence of LC3B phosphorylation, whereas, starvation induced a release of LC3B-FYCO1 interaction in phosphorylation-proficient cells, possibly to facilitate retrograde transport of APs. Collectively, these findings show that phosphorylation of LC3B regulates the directional transport of APs, which may be especially important

during starvation. Understanding how this molecular pathway is modified in disease and aging is an important future objective.

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POSTER NUMBER: 2043

Long-term synaptic depression triggers autophagosome biogenesis in dendrites and requires autophagic degradation

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It is well-established that in neurons, autophagosome biogenesis is spatially confined to the axon tip under baseline conditions. However, it remains unknown whether autophagy biogenesis can be induced in other neuronal compartments following synaptic activity. Here, we show that two major forms of long-term synaptic depression (LTD), mediated by activation of N-methyl-D-aspartate (NMDAR) or group 1 metabotropic Glutamate (mGluR) receptors, trigger the local translation of autophagy initiation complex components and rapid assembly of autophagosomes in dendrites. Abolishing this immediate autophagic response is sufficient to totally prevent LTD, suggesting that LTD critically relies on autophagic degradation of synaptic proteins. In line with this, animals with reduced brain autophagy exhibit impairment in cognitive functions that require LTD, such as behavioral flexibility and memory. Using quantitative proteomics in purified autophagosomes, we reveal that the autophagic cargo is enriched for post-synaptic density proteins, as well as modulators of the actin cytoskeleton and autism-implicated proteins after LTD. Thus, persistent synaptic depression requires the local synthesis and assembly of the autophagic machinery in dendrites, which ensures the elimination of synaptic structures via degradation of their components, facilitating plasticity and associated behaviors.

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POSTER NUMBER: 2044

A novel role for autophagy and the autophagosome in antisense oligonucleotide activity and trafficking

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Autophagy is an evolutionarily conserved membrane trafficking process which delivers cargo from the cytoplasm via double membrane autophagosomes to lysosomes and is critical for the breakdown and reuse of intracellular materials to maintain cellular homeostasis. Endocytosis is a mechanism by which cells sense their environment and uptake various nutrients, initiating at the plasma membrane, converging with autophagy, and terminating with the lysosome. Like many small molecules, antisense oligonucleotides (ASOs), broadly used as research tools and therapeutic agents, can be internalized via multiple endocytic pathways creating a complex web of intracellular traffic. The majority of intracellular ASOs colocalize with the lysosome, which serves as a “non-productive sink,” and only a small fraction that partakes in escape from late endosomes contributes to activity. As both autophagy and endocytosis have many converging steps and common molecules, it is unclear if early stages of autophagy-related trafficking participate in ASO uptake or how modulation of autophagy affects ASO activity and localization. To answer these questions, we subjected various cell lines and C57bl/6J mice to treatment with either Rapamycin or AZD8055, or nutrient deprivation via 16-hour fasting in order to activate autophagy prior to ASO administration. We found that enhancing autophagy significantly increased ASO knockdown activity dose-dependently both *in vitro* and *in vivo* with ASO free-uptake (10 hours) or subcutaneous ASO administration (24 hours), respectively. *In vitro* studies revealed that autophagy activation enhanced the preference and rate of ASO localization into autophagosomes, a compartment previously not associated with ASO trafficking. Moreover, these effects could be mitigated by inhibiting autophagosome formation prior to autophagy induction and ASO uptake. These enhancement effects were consistent across multiple ASO chemistries and gene targets and were not

a result of changes in the rate of ASO uptake, extracellular release, cellular concentration, or distribution into early endosomes or lysosomes within the cell. These results support a novel role for autophagy and specifically, the autophagosome, as a potential unidentified compartment that participates in and contributes to enhanced ASO activity and trafficking.

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POSTER NUMBER: 2045

Repression of autophagy and phosphorylation of autophagy regulators during mitosis is independent of mTORC1

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It has been proposed that macroautophagy must be repressed during mitosis otherwise nuclear envelope breakdown will expose the genome to the cytosolic autophagy machinery, which could cause DNA damage and genomic instability. However, evidence that autophagy is repressed during early mitosis remains controversial and mechanistic detail is limited to the suggestion that CDK1 phosphorylates VPS34. The nutrient-responsive mTORC1 kinase complex normally inhibits autophagy via repressive phosphorylation of the key autophagy regulators ULK1, ATG13, ATG14 and TFEB. Studies have suggested that the mTORC1 substrates S6K and 4E-BP1 are instead phosphorylated by CDK1 during mitosis, resulting in CDK1 taking over the regulation of translation. We therefore hypothesized that CDK1 might substitute for mTORC1 to enforce repression of autophagy during nuclear envelope breakdown. Here we show that autophagy initiation, as measured by ATG13 puncta formation in both live-cell and fixed-cell samples, is indeed repressed throughout mitosis. This occurs even when cells are treated with classical autophagy inducers (amino acid starvation or the ATP-competitive mTOR inhibitor AZD8055). Furthermore, autophagy regulators undergo mitotic hyperphosphorylation in a manner dependent on CDK1 activity but not mTOR or MEK-ERK1/2 signalling. Crucially, this includes the known repressive sites that are normally phosphorylated by mTOR in interphase cells. Thus, we conclude that mTORC1 is substituted, most likely by CDK1, as the master repressor of autophagy during mitosis. This uncouples autophagy regulation from nutrient status and maintains repression of autophagy during mitosis.

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POSTER NUMBER: 2046

Procollagen quality control occurs via non-canonical autophagy of modified ER exit sites

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Type I collagen is the main component of bone matrix and other connective tissues. Defects in procollagen folding, trafficking and degradation are key factors in bone and additional connective tissue pathologies. Autophagy has been identified as the degradation pathway for misfolded procollagen that avoids activation of generic ER-stress responses; however, the mechanism by which this occurs is unclear. To address this question, we used live cell and correlative light-electron microscopy (CLEM) imaging of procollagen with a Gly610-to-Cys substitution in mouse osteoblasts. This substitution mimics a mouse model of bone pathology that involves autophagic degradation of misfolded mutant procollagen. Cells expressing fluorescently tagged proteins revealed procollagen puncta co-localized with an autophagic membrane marker LC3. These puncta did not contain ER lumen or membrane proteins but did contain proteins associated with ER exit sites (ERESs), specifically components of the COPII coat. Procollagen autophagic puncta formation was decreased when ERES formation was inhibited, but disruption of later steps in the secretory pathway had no effect. This suggests rerouting of misfolded procollagen towards autophagy occurs at ERESs. We then analyzed whether traditional macroautophagy was involved in formation of puncta containing procollagen, LC3 and COPII proteins. Inhibition of autophagosome-lysosome fusion by bafilomycin A1 did not affect lysosomal internalization of these colocalized puncta. Furthermore, CLEM revealed that the lysosomal

membrane marker LAMP1 was not restricted to the lysosome surface, but instead was encapsulated within lysosomes, intermixed with procollagen, LC3 and COPII, indicating lysosomal engulfment rather than traditional autophagosome-lysosome fusion. Overall, our findings suggest that misfolded procollagen triggers modification of ERES membranes by autophagic machinery, which leads to the ERES being engulfed by lysosomes through a non-canonical, microautophagy pathway.

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POSTER NUMBER: 2047

K33-linked polyubiquitin is recruited by SQSTM1/p62

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Ubiquitin chain is a prerequisite site for substrate recognition and determines selectivity in autophagy. Ubiquitin chains are formed with 8 structurally and functionally distinct polymers. However, the functions of each polyubiquitin remain poorly understood. We examined bimolecular fluorescence complementation (BiFC) methods for polyubiquitin chain analysis. We developed a polyubiquitin-mediated fluorescence complementation (PolyUb-FC) assay using Kusabira Green (KG) as a split fluorescent protein. The PolyUb-FC assay has the advantage that monoubiquitination is nonfluorescent and chain-specific polyubiquitination can be directly visualized in living cells without using antibodies. We applied the PolyUb-FC assay to examine SQSTM1/p62. We demonstrated that SQSTM1/p62 puncta colocalized with PolyUb(WT)-FC. SQSTM1/p62 is thought to bind mainly through K63-linked and K48-linked polyubiquitin. Confocal microscopy revealed that PolyUb (K33)-FC puncta colocalized with SQSTM1/p62 puncta, however, PolyUb(K33)-FC did not colocalize with the SQSTM1/p62 UBA mutant. To determine the ability of K33-linked ubiquitin to bind SQSTM1/p62, we performed a cell-free assay and found that tetra K33 bound to SQSTM1/p62. Additionally, K33-linked polyubiquitin and this interaction was modulated by the ZRANB1/TRABID-K29 and -K33 linkage-specific deubiquitinase (DUB). We further showed that the colocalization of K33-linked polyubiquitin and MAP1LC3/LC3 (microtubule associated protein 1 light chain 3) puncta was impaired by SQSTM1/p62 deficiency. Taken together, these findings provide novel insights into not only K48- and K63-linked polyubiquitination, but also indicate that K33-linked ubiquitin is recruited by SQSTM1/p62.

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POSTER NUMBER: 2048

Insights into phagophore expansion from structural and biochemical analyses of the ATG2A-WIPI4 complex

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ATG2 is the largest member of the group of autophagy-related proteins and is one of the least characterized ones. A collection of previous studies has suggested that ATG2 plays a role in the expansion of the phagophore by localizing at the ER-phagophore junction. We have recently performed structural and biochemical analyses of human ATG2A-WIPI4 complex, which unveiled membrane-tethering activity of ATG2A. ATG2A is a rod-shaped protein and each of the two ends of the rod is able to bind to a membrane, enabling the protein to bridge membranes/vesicles. The autophagic PI3P-binding protein WIPI4 associates with one end of the rod, directing the WIPI4-bound end of the ATG2A rod to PI3P-containing membranes. Having the ability to bind specifically to a PI3P-membrane at one end, ATG2A can tether a PI3P-containing membrane to a non-PI3P-containing membrane. These physical properties of the ATG2A-WIPI4 complex obtained from our in vitro studies led us to propose that the ATG2A-WIPI4 complex tethers the omegasome to neighboring membranes.

The membrane-tethering activity by the rod-shaped ATG2A reminded us of tubule-shaped lipid transfer proteins and a recent report on Vps13, a protein that shares sequential and architectural similarities with ATG2A, showed that Vps13 is a lipid transfer protein. Thus, we have performed fluorescence-based lipid transfer assays on ATG2A, which revealed the lipid transfer activity of ATG2A. ATG2A can extract lipids from membranes and unload them to different membranes. ATG2A tethers small vesicles and transfer lipids efficiently between them but poorly between larger vesicles, which ATG2A cannot tether. When PI3P is included in large vesicles, WIPI proteins stimulate ATG2A-mediated lipid transfer between those PI3P-containing vesicles and non-PI3P-containing vesicles. Based on these findings, we propose that ATG2A-WIPI complexes not only tether the ER-phagophore junction but also mediate the transfer of lipids from the ER to the phagophore, providing a physical model for the expansion of the phagophore membrane.

 POSTER NUMBER: 2049

Autophagy Induction is Required to Enhance Cognition and Integrate the Effects of Youthful Systemic Factors in the Aged Brain

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Normal brain aging is characterized by a progressive decline in cognitive functions that develop around midlife. One of the most commonly affected regions of the brain is the hippocampus, which leads to learning deficits and memory impairments. With increased life expectancies in developed societies, the number of individuals affected by age-related memory loss is bound to dramatically increase. Therefore, a deeper understanding of how a healthy brain ages and the identification of cellular mechanisms involved in age-related cognitive deficits are now a highly-prioritized scientific challenge.

Age-related declines in cognitive fitness are associated with a reduction in autophagy, an intracellular lysosomal catabolic process that regulates protein homeostasis and organelle turnover. However, the functional significance of autophagy in regulating cognitive function and its decline during aging remains largely elusive.

Using hippocampal injections of genetic and pharmacological modulators of autophagy, we find that inducing autophagy in hippocampal neurons is required to form novel memory, by promoting the activity-dependent structural and functional synaptic plasticity, including dendritic spine formation, neuronal facilitation and long term potentiation. We show that promoting hippocampal autophagy in aged mice is sufficient to reverse age-related memory deficits. Lastly, we demonstrate that systemic administration of young plasma into aged-mice rejuvenates memory in an autophagy-dependent manner, suggesting a prominent role for autophagy to favor the communication between systemic factors and neurons in fostering cognition. Among these youthful factors, we identify Osteocalcin, a bone-derived molecule, as a direct hormonal inducer of hippocampal autophagy in old mice.

Altogether, these data demonstrate that the activation of autophagy is a necessary mechanism to promote novel memory formation and to favor the influence of youthful systemic factors on cognitive fitness.

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 POSTER NUMBER: 2050

MiT-TFE Transcription Factors Couple Autophagy and Metabolism to the Peripheral Clock

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Autophagy and energy metabolism are strongly controlled by the circadian clock. The biochemical basis for coordinated control of autophagy and the circadian clock is still under investigation. Here we show that the oscillation of autophagy genes is dependent on the activation of TFEB and TFE3, key regulators of autophagy, lysosomal biogenesis and cell homeostasis. TFEB and TFE3 display a circadian activation over the 24hour cycle and are responsible for the rhythmic induction of genes involved in autophagy during the light phase. Genetic ablation of TFEB and TFE3 in mice results in deregulated autophagy over the diurnal cycle and altered expression of the circadian genes causing abnormal circadian wheel-running behavior. In addition, we found that TFEB and TFE3 directly regulate the expression of *Rev-erba (Nr1d1)*, a transcriptional repressor component of the core clock machinery also involved in the regulation of whole-body metabolism and autophagy. Comparative analysis for the

cistromes for TFE3 and REV-ERB α showed an extensive overlap of their binding sites enriched in genes involved in autophagy and metabolic function, thus revealing a direct connection between nutrient and clock-dependent regulation of gene expression. In conclusion, our study sheds a new light on the crosstalk between autophagy, metabolism and circadian cycles.

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POSTER NUMBER: 2051

Lysosome mediated remodeling of the cancer proteome

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Pancreatic ductal adenocarcinoma (PDA) cells and tumors constitutively activate nutrient scavenging pathways namely autophagy (cellular self-catabolism) and macropinocytosis (bulk uptake of extracellular material) in order to maintain metabolic homeostasis. Importantly, both of these pathways converge on the lysosome, an acidic organelle that harbors within its lumen a complex series of enzymatic reactions that enable degradation of in-coming cargo material. Our prior work has shown that the lysosome is greatly expanded and qualitatively different in PDA cells compared to normal tissue. PDA tumors are effectively “addicted” to enhanced catabolic activity as blocking lysosome function specifically inhibits *in vitro* and *in vivo* growth of PDA cell lines and tumor xenografts with minimal effects on normal cells. How key changes in lysosome composition and content contribute to the aggressive nature of PDA remains an open question. We have optimized methodologies to isolate and purify lysosomes from normal human pancreatic epithelial cells and several established patient derived PDA cancer cell lines, using affinity based capture techniques that isolates 50-80% intact lysosomes free of cytoplasm and other contaminating organelles. By systematically implementing lysosome purification from normal, primary and metastatic PDA tumors combined with mass spectrometry based proteomics, we have identified both resident and substrate proteins that are uniquely associated with PDA lysosomes. Several cancer-specific lysosomal membrane proteins emerged from this analysis that may endow cancer lysosomes enhanced functionality including rapid membrane repair in the face of sustained mechanical and chemical insults. Similarly, we find that PDA lysosomes degrade select cellular proteins to enable dynamic remodeling of the cellular proteome in a manner that favors tumor growth and progression. Collectively, our data provide strong evidence in support of the lysosome as a central mediator of cellular adaptation to stress and promoter of tumorigenesis.

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POSTER NUMBER: 2052

Autophagy: a novel link between matrix vesicles and vascular calcification?

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Patients with end-stage renal disease (ESRD) have elevated circulating calcium (Ca) and phosphate (Pi), and exhibit accelerated progression of calcific aortic valve disease (CAVD). We have recently highlighted a critical role for matrix vesicles (MVs) in the initiation of the calcification process in CAVD, through amorphous calcium phosphate or crystalline hydroxyapatite matrix deposition. However, the origin of these matrix vesicles and their contents are poorly characterised. Proteomics analysis of MVs derived from calcified rat valve interstitial cells (VICs) has revealed significant enrichment of key macroautophagy and mitophagy proteins, including LAMP-1 (fold change 49.6), LAMP-2 (fold change 41.8), LAMTOR1 (fold change 8.023) and PARK7 (fold change 516.63). We therefore hypothesise that the early MVs with amorphous calcium phosphate traffic via the autophagosome - lysosomal pathway. Using (i) immunofluorescence and (ii) transmission electron microscopy (TEM) combined with energy dispersive x-ray spectroscopy in calcified rat VICs, we demonstrate that MVs enriched with calcium and phosphate are present within autolysosomes. Furthermore, our data reveals that inhibiting the formation of mature autophagosomes leads to increased VIC calcification using LY294002 (40 μ M), a PI3K inhibitor, ($P < 0.0001$).

Interestingly, our TEM data also reveals mitochondria as a source of calcium and active ongoing mitophagy in RVICs under calcifying conditions. These data suggest that autophagosomes may play a vital role in recycling the calcium and phosphate build-up in MVs. Our work proposes a novel link between autophagosomes and vascular calcification which could lead to the development of therapeutic strategies against CAVD.

 POSTER NUMBER: 2053

Autophagy maintains tumor growth through different mechanisms depending on neoantigen load

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Autophagy captures intracellular components and delivers them to lysosomes where they are degraded and recycled. Cancer cells benefit from autophagy, as loss of autophagy impairs the metabolism, proliferation, survival and malignancy of tumors in a variety of autochthonous cancer models. Moreover, acute, systemic deletion of *Atg7* in mice induces far greater regression of Kras-driven lung cancer than tumor-specific deletion, suggesting a role for host autophagy in promoting tumor growth. Here we show that host-specific *Atg7* deletion dramatically impaired tumor growth and identify two mechanisms involved in this process: 1) by depleting circulating arginine for low neoantigen load tumors or 2) by promoting a T cell response for high neoantigen load tumors. For low neoantigen load tumors, we show that host autophagy prevents release of Arginase 1 from hepatocytes, preserving the levels of circulating arginine essential for the growth of arginine auxotrophic tumor (lacking argininosuccinate synthase). Supplementation of *Atg7* Δ/Δ hosts with arginine partly restores serum arginine levels and tumor growth, identifying a novel metabolic vulnerability of cancer (Nature, in press). For high neoantigen load tumors, *Atg7* Δ/Δ hosts more efficiently reject tumors than *Atg7*^{+/+} hosts, which is rescued by T-cell depletion. Expression of immune-related genes are increased in tumors from *Atg7* Δ/Δ hosts in a T-cell dependent manner. Tumors from *Atg7* Δ/Δ hosts also have decreased T-regulatory (T-reg) cells and depletion of these cells is able to mimic the reduced tumor growth observed in the *Atg7* Δ/Δ hosts, suggesting that autophagy modulates tumor growth through T-reg dependent mechanism. Altogether, our results identify two mechanisms used by autophagy in the host to regulate tumor growth depending on the neoantigen load.

 POSTER NUMBER: 2054

ASO-dependent knockdown of USP30 enhances mitophagy and protects against acute mitochondrial injury *in vivo*

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Mitochondria are exquisitely dynamic organelles that perform essential cellular biosynthetic reactions including production of ATP, amino acids, nucleotides, iron-sulfur clusters and various metabolic intermediates. It is widely believed that perturbations in mitochondrial function underlie many age-related diseases and that autolysosomal degradation of damaged mitochondria - termed mitophagy - counteracts dysfunction to maintain cellular health. However, development of therapeutic agents that specifically enhance mitophagy has remained elusive owing to an incomplete understanding of the pathway. Recently it was shown that ubiquitin-specific peptidase 30 (USP30) directly opposes mitophagy by suppressing the recruitment of autophagic machinery, and that knockdown of USP30 improves mitochondrial network integrity *in vitro* and *in vivo* by increasing mitophagy. Here we tested the hypothesis that antisense oligonucleotide (ASO)-mediated knockdown of USP30, which specifically modifies gene expression through RNase H1-mediated degradation of USP30 RNA, enhances mitophagy under basal- and injury-induced conditions. ASOs targeting USP30 elicited robust knockdown of USP30 RNA in tissue culture cells stably expressing the mCherry-GFP-Fis1(101-152) mitophagy reporter. In addition, we found that USP30 depletion led to a significant increase in the number of red puncta, indicating an increase in mitochondrial localization to lysosomes. To determine if enhanced mitophagy is protective *in vivo* following acute mitochondrial injury, we dosed C57BL/6 mice subcutaneously with USP30 ASO for 4 weeks and then challenged with 500 mg/kg acetaminophen (APAP). ASO treatment elicited >80% reduction in liver USP30 RNA and significantly increased autophagy as indicated by increased LC3B lipidation and decreased Ndp52, Optineurin and Tax1bp1. Whereas mice treated with PBS or non-targeting control ASO exhibited robust increases in ALT and AST following APAP challenge, we observed that mice treated with USP30 ASOs were protected from APAP-induced injury. These results demonstrate that ASO knockdown of USP30 enhances mitophagy *in vitro* and protects against acute mitochondrial

injury *in vivo* and highlight the therapeutic potential of USP30 ASOs in treating conditions associated with deficits in mitochondrial function.

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POSTER NUMBER: 2055

Autophagy regulates Rac1GTP and RhoAGTP activity in dendritic cells and intestinal epithelial cell lines

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The SNP (T300A) in the ATG16L1 gene reduces autophagy and is one of the few highly prevalent risk factors associated with Crohn's disease. We have previously shown that reduced autophagy results in enhanced dendritic cell (DC) - T cell contact time and T cell hyperactivation. Furthermore, we have shown that autophagy^{low} DCs have altered cytoskeletal morphology and exhibit a reduced capacity to migrate. RhoGTPase Rac1^{GTP} activity was enhanced in autophagy^{low} DC, and morphology and migratory capacity were restored upon Rac1^{GTP} inhibition. Here we further investigated the role of autophagy and its effect on RhoGTPase modulation in DCs, and as the T300A SNP is a body wide phenomenon, in two intestinal epithelial cell lines.

Methods Autophagy^{low} DCs and cell lines (Caco-2 and HT29) were generated using siRNA and lentiviral shRNA respectively. ATG16L1 protein knockdown was confirmed on Western Blot. Rac1^{GTP} and RhoAGTP activity was measured using G-Lisa. Migration capacity of Caco-2 and HT29 cell lines was determined using a scratch assay. Proliferation was analyzed using EdU incorporation.

Results In contrast to the increase of Rac1^{GTP} activity, RhoAGTP activity was decreased in autophagy^{low} DCs (n=10 individual donors). Similarly, in both Caco-2 and HT29 autophagy^{low} cell lines Rac1^{GTP} activity was increased and RhoAGTP was decreased compared to the respective control cell lines. Whole protein levels of Rac1 and RhoA however, were not altered, suggesting the regulation is not translational. In scratch assays, the autophagy^{low} Caco-2 and HT29 cell lines had reduced capacity to close the wound compared to the control cell lines, thus implicating reduced migratory capacity, similar to that found in DCs. This was not due to a decrease in proliferation in the autophagy^{low} cell lines.

Conclusion Reduced autophagy in both DCs and epithelial cell lines causes an increase in Rac1^{GTP} activity and decrease in RhoAGTP activity. These results suggest that a defect in the autophagosomal regulation of Rac1^{GTP} and RhoAGTP underlies the association between ATG16L1 T300A SNP and Crohn's disease, which impacts both immune and epithelial wound healing responses.

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POSTER NUMBER: 3001

The Initiation of Ubiquitin-Independent Mitophagy by Atg32

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The selective autophagy of mitochondria, termed mitophagy, leads to the degradation of damaged or excess mitochondria. Mitochondria to be degraded are identified by mitophagy receptors. These receptors are divided into two distinct classes. The first set of receptors are cytosolic proteins that recognize ubiquitin moieties on mitochondrial proteins. The second set of mitophagy receptors are resident outer mitochondrial membrane proteins that initiate mitophagy independent of ubiquitin. Insight into the initiation of ubiquitin-independent mitophagy has been hindered by a lack of structural data on these outer mitochondrial membrane mitophagy receptors. In *Saccharomyces cerevisiae*, ubiquitin-independent mitophagy is initiated by Atg32, a 529 amino acid single pass outer mitochondrial membrane protein. Atg32 contains a cytosolic domain of 380 amino acids, a single transmembrane helix, and a 120 amino acid domain residing in the inner membrane space of mitochondria. We have utilized NMR spectroscopy, small angle X-ray scattering and molecular dynamics simulations to dissect the structure of the N-terminal cytosolic portion of Atg32. This structural work revealed that the cytosolic portion of Atg32 contains both structured and highly dynamic regions. The structured portion of Atg32 contains a pseudo-receiver domain that is required for Atg32 activation. The dynamic portion of Atg32 supports interactions with Atg11 and Atg8. Both the structured and dynamic regions of Atg32 function together to facilitate Atg32 activation and mitophagy induction. We have also investigated the activation mechanism of Atg32 in cells. Taken together, this

work has revealed a complicated mechanism for the activation of Atg32 that requires communication between the cytosolic and inner membrane space regions of Atg32.

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POSTER NUMBER: 3003

Mitochondrial malfunction impairs lysosomal hydrolysis via AMPK-PtdIns(3,5)P₂

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Mitochondrial dysfunction and impaired autophagy are often associated. Defective autophagy can often result in the accumulation of impaired mitochondria in the cytoplasm. However, it remains poorly understood if mitochondria can also impact autophagy. We have found a novel pathway by which long-term mitochondrial malfunction, such as observed in mitochondrial diseases or in aging, triggers a signaling network that culminates in the impairment of lysosomal functions and blockage of the autophagy pathway.

We show here that chronic mitochondrial stress (respiratory chain disruption by stable silencing of a complex III subunit or by knock-out of a complex I subunit) impairs lysosomal Ca²⁺ homeostasis, resulting in lysosomal Ca²⁺ accumulation and swelling, impaired lysosomal acidification and catabolic activity, and stalled autophagy. These effects on lysosomal function are independent of autophagosome delivery. Lysosomal function can be rescued by reactivation of the MCOLN1/TRPML1 lysosomal Ca²⁺ channel. The decrease in MCOLN1 activity under chronic mitochondrial defects is due to a decrease in AMPK activity. Accordingly, reactivation of AMPK also restores lysosomal function. Notably, AMPK α 1 α 2 DKO fibroblasts present defects in lysosomal function similar to chronic mitochondrial malfunction, which can be also be rescued by MCOLN1 reactivation. The decrease in AMPK signaling under chronic mitochondrial dysfunction is mediated by the up-regulation of the AMPK inhibitor protein folliculin. Silencing of folliculin in cells with chronic mitochondrial stress restores lysosomal morphology, acidification and function, as well as the autophagy pathway.

These results show that chronic mitochondrial deficiency exerts a regulatory inhibition on lysosomes and on the autophagy pathway, and show that AMPK regulates lysosomal physiology, including biogenesis and function.

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POSTER NUMBER: 3004

Investigating the Role of Specific VPS34 Complexes

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Vps34 is the sole representative of the class III PI3Ks, and generates PI3P to regulate intracellular trafficking such as endosomal sorting, macroautophagy, and phagocytosis.

Vps34 forms mainly two heterotetrameric complexes, known as complex I and II, which control autophagy and endosomal maturation respectively. The core complex consists of Vps34, Beclin1 and Vps15, and associates in a mutually-exclusive manner with either ATG14 or UVRAG to form complex I or II.

In order to gain a deeper understanding on how Vps34 regulates autophagy and endocytosis, we aim to develop nanobodies that bind specifically to each of the Vps34 complexes as a tool to track in cells the dynamic of each complex under different stimuli. Current ongoing work on cell lines allowed us to characterize complex I specific nanobodies, and further functional analysis will help us to identify new roles of complex I and whether it functions beyond its role in autophagy.

POSTER NUMBER: 2060

Structural and biochemical characterization of the FYVE domain of ALFY

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The accumulation of misfolded or aggregated proteins is a hallmark of many neurodegenerative diseases. Selective autophagy mediates the clearance of protein aggregates in order to maintain cellular homeostasis. Autophagy-linked FYVE protein (ALFY), a large scaffolding protein, plays an essential role in the degradation of misfolded and aggregated proteins by linking cargo laden receptors to the autophagic machinery. ALFY contains a FYVE domain at its C-terminus, which has been shown to bind to phosphatidylinositol 3-phosphate (PI(3)P) found in the autophagic membrane. To gain additional insight into the FYVE domain of ALFY, we have obtained a structure of the FYVE domain using nuclear magnetic resonance (NMR) spectroscopy. We have also probed the lipid binding properties of this domain using lipid sedimentation assays. These experiments have provided new insight into the mechanism of membrane targeting by the FYVE domain of ALFY.

POSTER NUMBER: 3005

Host defence peptide LL-37 activates autophagy to control intracellular bacterial survival in macrophages

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Autophagy is an intracellular degradation system that delivers cytoplasmic constituents to the lysosome, and has important physiological and pathophysiological roles. We have previously shown that phenylbutyrate (PBA) and/or vitamin D3 (vitD3) activate the autophagy process and subsequently kill *Mycobacterium tuberculosis* (*Mtb*) in macrophages via the host defence peptide LL-37. Thus, our overall aim was to investigate the detailed mechanisms of PBA and vitD3-mediated effects on LL-37 dependent autophagy in macrophages with a special focus on the identification of receptors, signalling pathways and transcription factors involved. LL-37 peptide alone was able to induce autophagy and improve the killing of *Mtb* in macrophages. Notably, LL-37 activated autophagic flux, as shown by the marker SQSTM1/p62 (sequestosome 1) protein, which is an ubiquitin-binding scaffold protein that is degraded by autophagy. Here, the SQSTM1 protein was detected in untreated macrophages but the levels decreased significantly in LL-37 treated macrophages. Further, we demonstrated that the enhanced autophagy by LL-37 was mediated through the P2X7 receptor (a purinergic receptor) found on the surface of macrophages. Additionally, intracellular free Ca^{2+} , adenosine monophosphate-activated protein kinase, and phosphatidylinositol 3-kinase pathways were involved in LL-37-mediated activation of autophagy in human macrophages. Finally, treatment with PBA and VitD3 resulted in binding of the transcription factor EB (TFEB) to the promoter of the *CAMP*-gene encoding LL-37 as shown by chromatin immune-precipitation (ChIP) analysis. In conclusion, our results suggest that LL-37 activates TFEB, a key transcription factor for autophagy, which controls the major steps of the autophagy pathway and subsequently improves antimicrobial effects against intracellular bacteria.

POSTER NUMBER: 3007

Increased HSP25 triggers remodeling of collagen and augments lysosomal function in a worm model of neurons-specific tauopathy

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Both aging and many neurodegenerative disorders are characterized by protein aggregates, which may arise or be exacerbated by a failure of the protein homeostasis network. Small heat shock proteins (sHSPs) are molecular chaperones that function not only in protein folding, but also improve the degradation activity of the proteasome and autolysosome, decreasing disease-associated aggregates. Previous work from our lab has shown that the expression level of small heat shock protein 25 (HSP25) in muscle and liver tissue correlates best with maximum lifespan potential in rodents, yet how this chaperone improves lifespan and the effect it may have on protein aggregation in neurodegenerative disease is unknown.

To explore the role of HSP25 in longevity, we created a nematode overexpressing HSP25 from the naked-mole rat, and found that HSP25 improves lifespan potential and heat resistance in *C. elegans*. RNAi experiments suggest that the lifespan extension is dependent on *hsf-1* and *skn-1*, but independent of *daf-16*. RNAseq from HSP25 overexpressing worms show enrichment in several SKN-1/Nrf2 associated pathways, such as collagen proteins, the innate immune response, and lysosome function. Increased HSP25 also has a beneficial effect on proteostasis in a *C. elegans* neuron-specific tauopathy model, as indicated by an increase in lifespan and heat resistance and a decrease in protein aggregation. We are currently working to mechanistically define the effect of high levels of HSP25 protein in these models and understand the role of increased lysosome function and its contribution to aggregation mitigation in this worm strain.

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POSTER NUMBER: 3008

Structural basis and in situ visualization of p62/SQSTM1 polymers by electron microscopy

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Selective autophagy is the mechanism by which large molecular cargos of various sizes from molecules, organelles to pathogens are specifically sequestered for degradation in the lysosome. We determined a high resolution cryo-EM structure of p62-PB1 filaments revealing the molecular basis of polymer formation and relate these findings with tomographic reconstructions of cellular p62 bodies. It has recently emerged that autophagy receptors support the selective sequestration and transport of specifically marked cargo to nascent autophagosomes [1]. In this process, autophagy receptors recognize ubiquitylated cargo and interact with Atg8/LC3 to bring the cargo-receptor complex in contact with the autophagosomal membrane. The structural details of cargo and receptor assembly giving rise to autophagic vesicles remain to be elucidated. Recently, we showed that autophagy receptor p62/SQSTM-1 assembles into flexible helical polymers [2]. Here, we used recent advances in cryo-EM to generate a near-atomic resolution helical reconstruction of p62-PB1 filaments. Using EM based structure elucidation in situ we show that large oligomeric and polymeric cargo receptor complexes are the major component of p62 bodies inside of cells and define the architectural requirements of these assemblies for their cargo uptake and degradation using cellular turnover assays. The organization of small receptor proteins into helical polymers provides a cellular mechanism for high selectivity in cargo recognition and a fundamental architectural scaffold enabling cargo encapsulation.

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A novel drug molecule induces p62 oligomerization and abrogates cisplatin induced autophagy mediated resistance process in human cancer models

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Cisplatin (CDDP) is used as a prominent first line drug against solid tumors, either alone or in combination with other anticancer agents. However, while CDDP induced DNA damage and ER stress govern initial disease sensitivity, adaptation to stress by upregulation of pro-survival mechanisms, such as autophagy, may underpin resistance to CDDP and remains a stumbling block to successful outcome. P62 acts as a receptor or adaptor for autophagic degradation of ubiquitinated proteins, and plays an important role in preventing ER stress-induced apoptosis, leading to CDDP resistance in cancer. By screening an in-house library of stilbene analogues, we report here the first orally available small molecule, BCTAR, having the abilities to induce p62 oligomerization and simultaneous autophagy inhibition. We showed that BCTAR functions through rapid induction of p62 oligomerization (with the oligomers mostly present in the detergent insoluble fraction of cells) and inhibition of autophagosome formation as well fusion with lysosome. More interestingly, BCTAR mediated p62 oligomerization was further enhanced in presence of CDDP, putatively resulting in CDDP cross-linked p62 aggregates which were resistant to cellular protein degradation systems even after removal of the drugs, and may be one of the underlying cause of combination treatment induced sensitisation. ICP-MS data revealed the presence of significant amount of CDDP in p62 aggregates and supports the above hypothesis. An *in vitro* cell free assay revealed that BCTAR can act directly on p62 and generate detergent-insoluble aggregates. CDDP induced autophagy up-regulation was primarily due to activation of RAS-RAF-ERK signalling pathway, but operates in an mTOR independent manner. However, mTOR was found to play significant role in survival of CDDP treated cells and concomitant inhibition of both of these pathways was responsible for the BCTAR mediated-potential of CDDP cytotoxicity. BCTAR also showed remarkable potential in sensitizing CDDP resistant subline of IMR32 cells. *In vivo* SCID mice data showed that the BCTAR-CDDP combination was highly effective in reducing neuroblastoma tumor burden. Overall, our results revealed that BCTAR can be used as an adjuvant with CDDP in managing neuroblastoma.

HIV-1 Vpr Leads to alpha-synuclein aggregation

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Even with the advancement in HIV treatment with cART (combinatory anti-retroviral therapy), HIV-associated neurocognitive disorders (HAND) exist as a major problem among HIV-1 infected patients. HIV-1-associated dementia (HAD) and related cognitive and motor disorders affected 20%-30% of patients with advanced immunosuppression or AIDS.

Increased α -synuclein in the substantia nigra, a hallmark of Parkinson's disease, was recently reported in HIV infection in older patients but not age-matched healthy individuals. Among older HIV+ adults, the frequency of α -synuclein is higher than that found in older healthy persons and may predict an increased risk of developing a neurodegenerative disease.

Accumulation of α -Synuclein is normally associated with its aggregation, increased toxicity, and alteration of neuronal communication leading to spatial memory loss and premature brain aging. The presynaptic protein α -Syn has a central role in Parkinson's disease (PD) and Lewy body disease. However, the mechanism by which the protein contributes to neurodegeneration and its normal function is not fully understood. Hence, we will determine the mechanisms used by HIV-1 Vpr leading to α -Syn aggregation.

Currently, although the precise aggregation mechanisms of alpha-synuclein are still unclear, overwhelming evidence demonstrates that oxidative stress plays a central role. Our previous results show that Vpr induces reactive oxygen species by increasing H₂O₂ production in microglia. The importance of HIV-induced oxidative stress on alpha-synuclein aggregation remains to define.

The HIV-1 virus is known to manipulate the cell autophagy; we also propose to use Rapamycin, a stimulator of autophagy, to contain Vpr damaging effect. The maturation of phagosomes, defined by the presence of late endocytic markers, hydrolases, and reactive oxygen species, is perturbed in HIV-1-infected macrophages. It was previously described that the viral regulatory protein (Vpr) was crucial to perturb phagosome maturation. We will investigate the relationship between the disturbance of the pathways autophagy/phagosomes by HIV-1 Vpr and alpha-synuclein aggregation.

 POSTER NUMBER: 3011

K63-polyubiquitin signaling in autophagy-related vesicular trafficking

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Several studies have indicated a role for the signaling molecule ubiquitin (Ub) in various forms of autophagy where the Ub modifies substrates for recognition by cargo receptors. However, the types of polyUb formed and their function in autophagy are not fully understood. We have investigated K63-linked polyUb's role in autophagy by using Vx3, a genetically-encoded probe we previously described that detects and inhibits K63-linked polyUb-dependent signaling [Sims et al., Nat. Methods (2012)], thereby allowing for tracking and isolation of K63-polyUb conjugates in cells. Vx3 expression in HeLa or MEF cells inhibits selective autophagy, such as autophagy of invading *Salmonella* bacteria, but not starvation-induced autophagy. However, Vx3 does not appear to have its effect by competition with autophagy receptors known to recruit LC3-II to cargo. In the absence of an autophagy trigger, Vx3 forms cytosolic foci that co-localize with late endosome/lysosome markers as well as two transmembrane proteins, ATG9 and VMP1, which have been reported to associate with membranes during early autophagosome biogenesis. Ultrastructural analysis revealed that these foci are comprised of small vesicles that cluster at the ER:endosome interface, suggesting that Vx3 inhibits fusion or trafficking of vesicles that share a common origin with autophagosomes. We have used affinity isolation and LC-MS/MS to identify K63-polyUb modified proteins associated with these vesicles. Surprisingly, these Ub-protein conjugates originate from newly-synthesized proteins en route to late endosomes/lysosomes in a Golgi-independent pathway. Our results shed light on new roles of K63-polyUb in the regulation of autophagy-related vesicular trafficking that extend beyond cargo recognition.

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 POSTER NUMBER: 3012

The Role of GSDMA3 on Autophagy in Models of Experimental Asthma

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Rational: Asthma is the most common chronic inflammatory disease. Recently, a gain-of-function mutation in Gasdermin A3 (GSDMA3) was shown to regulate autophagy in mice. GSDMA3 is one of three orthologues (GSDMA1-3) to human *GSDMA* located within the most replicated asthma susceptibility locus, 17q21. In a transgenic mouse strain (tgGSDMA3) carrying GSDMA3 mutations similar to those shown to alter autophagy, functional investigations were undertaken to link GSDMA3, autophagy, and allergen-induced airway dysfunction.

Methods: Intranasal [i.n., house dust mite (HDM)/alum] or intraperitoneal [ovalbumin (OVA)/alum] sensitization of tgGSDMA3 and wild-type (WT) mice was carried out on days 1 and 14. Sensitized or naïve mice received i.n. (HDM) or aerosol (OVA) challenges on days 24, 25 and 26. CD4⁺ T cells from HDM- or OVA-sensitized tgGSDMA3 and WT mice were isolated and adoptively transferred into sensitized WT or tgGSDMA3 mice. Lung allergic responses were determined. Gene expression of *Gsdma3* and autophagy genes (*Atg5*, *Atg7*, *Lc3a*, *Lc3b*, *Tfeb*) and proteins (GSDMA3, LC3-I/II, BCL2) were measured in the lung and isolated CD4⁺ T cells.

Results: In tgGSDMA3 mice, we identified missense mutations in GSDMA3. These mutations correlated with increased *Gsdma3* gene expression and markers of autophagy. In both allergen-induced models of experimental asthma, tgGSDMA3 mice failed to develop airway hyperresponsiveness with limited eosinophilic inflammation which was restored after adoptive transfer of allergen-sensitized WT CD4⁺ T cells. Adoptive transfer of tgGSDMA3 CD4⁺ T cells into allergen-sensitized WT mice, decreased lung allergic responses. Expression of autophagy genes in CD4⁺ T cells were significantly down-regulated in response to allergen sensitization and challenge in WT mice. Furthermore, BCL2 protein levels, one of the inhibitory molecules during autophagy, were elevated in CD4⁺ T cells from sensitized and challenged WT mice suggesting that autophagy down-regulation may be tied to asthma development.

Conclusion: These data provide the first link between mutations in *GSDMA3*, autophagy, and asthma pathogenesis.

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POSTER NUMBER: 3013

VPS13D is a Novel Regulator of Autophagy

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Defects in autophagy, the self-degradation of cellular components, are linked to multiple disorders such as cancer, diabetes and neurodegenerative diseases. Autophagosomes, containing cargo marked for degradation, fuse with lysosomes to recycle cell resources, such as protein aggregates and damaged organelles. However, we know little about the mechanisms that regulate the association between autophagic cargoes and autophagosome formation.

Here, I investigate the role of *vps13d*, an essential and widely conserved gene with relatively unknown function that is implicated in various human diseases such as movement disorders, septic shock, schizophrenia and cancer. Previous studies identified that VPS13D was crucial in context-specific autophagy required for cell death in *Drosophila* intestines. I now provide evidence linking VPS13D to the core autophagy machinery. Intriguingly, VPS13D also appears to be involved in dissolution of membrane contacts, which is associated with autophagosome formation, and requires a ubiquitin binding domain on VPS13D. Thus, VPS13D not only plays a very unique role in health and development, but may also explain the poorly understood role of ubiquitin in membrane contact regulation during autophagy.

I hypothesize that VPS13D facilitates context-dependent autophagy by associating ubiquitinated cargo with the autophagic machinery and disassembling membrane contact sites at the phagosome assembly site. Here I present data suggesting that VPS13D may function as a novel autophagy receptor for ubiquitinated cargo. Furthermore, VPS13D may facilitate autophagosome formation in a manner that is different from previous models involving ubiquitin receptors. The association of VPS13D mutations with human diseases illustrates the importance of studying and identifying the function of VPS13D.

POSTER NUMBER: 2061

Systematic analysis of the assembly of ULK1 complex

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Autophagy is a conserved and essential eukaryotic cellular process that degrades cellular organelles and other macromolecules via the lysosome. Although ULK1 complex, including ULK1, FIP200, ATG13 and ATG101, was identified as the initiator of autophagosome for years, the structural basis behind the assembly and catalytic regulation of this complex is still largely unknown. Here by using HDX-MS, we systematically identified regions in ULK1 complex which mediate complex assembly. Future work will be focused on more detailed structural analysis ULK1 complex by using Cryo-EM and XL-MS.

POSTER NUMBER: 3014

New Insights into the Mechanism and Biological Roles of Atg5-independent Macroautophagy

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Autophagy is morphologically characterized by double-membrane autophagosomes and single-membrane autolysosomes that degrade subcellular constituents using lysosomal enzymes. Autophagic membranes originate from mitochondria-associated ER membranes, and are elongated by Atg5-Atg12 and LC3 family members to generate autophagosomes. Recently, this machinery was reported to work slowly even in the absence of Atg5. On the other hand, we previously found an alternative type of autophagy, namely, alternative autophagy, which is generated by the curling of the trans-Golgi membrane. This autophagy is regulated by Ulk1, Dram1, and Rab9, but not by Atg5, Atg7, and LC3 family members. Basically, these two autophagic pathways degrade different molecules, and alternative autophagy plays a role not only in the genotoxic stress response, but also in mitochondrial elimination from differentiating reticulocytes.

Recently, we clarified the detailed molecular mechanism of alternative autophagy, in which Ulk1 phosphorylation at a yet-unidentified site plays an essential role at the trans-Golgi. We also identified the upstream kinase of Ulk1, which plays a role during alternative autophagy. This phosphorylation is not involved in Atg5-dependent autophagy. We also identified the mechanisms as to how autophagosomes are generated from the trans-Golgi membrane, in which alterations in local PI4P levels are involved. In this meeting, by describing some of these data together with more recent data, I will discuss the molecular mechanisms and physiological roles of alternative autophagy.

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POSTER NUMBER: 3015

Angiotensin II impairs autophagy and inefficiently removes abnormal/damaged and dysfunctional mitochondria in mouse skeletal muscle

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Introduction: Macroautophagy/autophagy is finely regulated to maintain basic intracellular functions, and either an excessive or defective activation of autophagy may disrupt skeletal muscle homeostasis. Angiotensin II (Ang II) activates the ubiquitin proteasome-system and disrupts energy metabolism in skeletal muscle. However, how Ang II regulates autophagy in skeletal muscle remains elusive. Our goal was to investigate the effects of Ang II on autophagy in mouse skeletal muscle. **Methods:** Ang II was infused (1.0 µg/kg/day) in male FVB mice (12 weeks-old) for 7 days; pair-fed (PF) group was infused with saline and served as control. Hindlimb muscles were used for western blot, RT-qPCR, mitochondrial respiration assay, and for transmission electronic microscopy (TEM). **Results:** Ang II reduced mouse body weight (12%, p=0.0001) and caused skeletal muscle loss (p<0.05). Herein, we found that Ang II blunted the LC3B-II conversion and increased p62/SQSTM1 protein expression. Ang II inhibited phosphorylation of ULK1 at Ser317 (p-ULK1), which led to a reduction in p-beclin1 (Ser14) and p-ATG14 (Ser29), critical VPS34 complex components that triggers autophagosome formation. Consistent with Ang II suppression of autophagy, TEM micrographs revealed that Ang II induced an accumulation of mitochondria with abnormal cristae and swollen morphology. These changes were associated with increased PINK1 protein expression. Importantly, Ang II markedly reduced the oxygen consumption rate in isolated mitochondria. These data indicate that Ang II impairs mitochondrial morphology and function. **Conclusion:** Our study indicates that Ang II negatively impacts the autophagosome formation. Moreover, consistent with Ang II-induced defective of autophagy, it induced an accumulation of abnormal/damaged and dysfunctional mitochondria. Our data strongly suggest that autophagy is critical to promote skeletal muscle homeostasis in Ang II-infused mice and raises the possibility of a new therapeutic target in wasting disorders such as heart failure.

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POSTER NUMBER: 3016

The role of chaperone-mediated autophagy on mechanotransduction in myoblasts

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Skeletal muscle has a remarkable ability to regenerate after injury because of resident muscle stem cells (SCs). SCs are located between the multinucleated muscle fiber and the extracellular matrix, which both provide intimate biophysical signals that guide SC behavior. After muscle injury, the SC microenvironment stiffness increases, which can persist even after muscle regeneration. Stiffness is sensed by mechanotransduction pathways, such as the transcriptional coactivator, YAP. *In vitro*, YAP localizes to the nucleus and regulates stem cell fate on stiffer environments. YAP is expressed in activated and proliferating SCs; however, the mechanism that controls its stiffness-dependent nuclear localization is unknown. Recently, chaperone-mediated autophagy (CMA) was found to be upregulated in muscle after injury and elevated on stiffer substrates. The objective of this research is to understand how CMA and mechanosensing pathways contribute to SC function using novel hydrogels that can stiffen upon light irradiation. *In situ* stiffening leads to an increase in YAP nuclear localization comparable to levels in myoblasts seeded onto pre-stiffened hydrogels, which are both significantly more nuclear than cells on soft hydrogels. Using small molecule inhibitors of autophagy, the YAP nuclear shuttling was diminished. We hypothesized that, by inhibiting CMA via impairing BAG3 function, CMA complex cannot bind to the cytoplasmic inhibitors of YAP, sequestering YAP in the cytoplasm. To target this BAG3-mediated mechanism, we knocked down (KD) BAG3 via siRNA and shRNA. BAG3 KD revealed downregulation of nuclear YAP targets, CTGF and ANKDR1, as well as YAP cytoplasmic retention via immunofluorescence independent of substrate stiffness. Furthermore, myoblast substrate-dependent proliferation is reduced through BAG3 KD. In conclusion, CMA complex assists YAP nuclear translocation, which can change the mechanically-driven myoblast behavior.

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POSTER NUMBER: 3018

TFEB regulates apoptosis and DNA repair independent of the lysosome in breast cancer cells

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Doxorubicin (DOX) is the standard of care treatment for triple-negative (TNBC) and hormone-therapy resistant breast cancer. Increased incidence of chemoresistance at lower doses and toxicity at higher doses limit the usefulness of DOX. By targeting pathways of chemoresistance, DOX can be an efficacious antitumor agent at lower doses. Lysosomal autophagy promotes resistance to several chemotherapies by guarding cells from cytotoxic metabolites and enabling tumour cells to scavenge cellular materials to accommodate increased levels of proliferation and energy production. Since transcription factor EB (TFEB) is a significant regulator of lysosomal biogenesis and autophagy in lung, renal and pancreatic carcinoma, we hypothesized a causal role for TFEB in breast cancer chemoresistance. In MDA-231 and BT549 TNBC cells, TFEB was hypo-phosphorylated and localized in the nucleus upon DOX treatment which corresponded with increased autophagy, lysosomal proteolysis and biogenesis. Knockdown of TFEB was sufficient to prevent DOX-induced autophagy activation and significantly sensitized MDA-231 and BT549 cells to DOX-induced apoptosis and loss of viability. Loss of TFEB function significantly slowed the repair of damaged DNA as measured by increased H2A.X phosphorylation 4 hours after DOX treatment. Notably, we find that the anti-apoptotic and DNA repair functions of TFEB are independent of lysosomal function. Finally, we show that calcineurin inhibition with cyclosporine A sensitized cells to DOX-induced apoptosis in a TFEB dependent fashion. Overall our data demonstrate that independent of the role of TFEB in regulating lysosome function, targeting TFEB in cancer cells decreases DNA damage repair rendering TNBC cells more sensitive to DOX at lower doses.

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POSTER NUMBER: 2062

Inhibition of Autophagosome-Lysosome Fusion Reveals New Protein Targets and Pathways of LC3-Dependent Secretion

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Autophagy is traditionally viewed as a degradative pathway in which cytoplasmic material is sequestered in double-membrane organelles called autophagosomes (APs) and delivered to the lysosome for breakdown. However, emerging evidence implicates the autophagy machinery in promoting unconventional secretion of proteins. We have uncovered that the autophagy conjugation machinery specifies the loading and secretion of proteins interacting with the autophagy regulator microtubule-associated protein 1 light chain 3B (LC3) in small extracellular vesicles (EVs), also termed “exosomes”. Both EV secretion and autophagic degradation contribute to cellular homeostasis, though the mechanism by which the two pathways coordinate regulation and cargo selection is unclear. Pharmacological inhibition of lysosomal acidification by bafilomycin A1 (BafA1), which blocks AP-lysosome fusion and therefore cargo degradation, increases the secretion of LC3 as well as autophagy cargo receptors (p62, NBR1, OPTN, NDP52) in EVs. Cargo receptors selectively deliver cargo to the growing AP during degradative autophagy by interacting with LC3, but have not previously been implicated in secretory autophagy. Similar to BafA1 treatment, genetic knockdown of two separate soluble NSF attachment protein receptor (SNARE) proteins mediating AP-lysosome fusion increases secretion of LC3 and cargo receptors in EVs. Mechanistic analysis reveals the autophagy conjugation machinery (ATG7, ATG12) is necessary for the secretion of cargo receptors. The further requirement of AP initiation (FIP200, ATG14) and closure machinery (ATG2A/B) for cargo receptor secretion indicates a novel mechanism for LC3-dependent secretion, potentially implicating the AP itself in the biogenesis of EVs carrying these cargo receptors. Overall, these findings establish a new relationship between degradative autophagy and LC3-dependent secretion, in which the impaired degradation of autophagic cargo due to the lack of AP-lysosome fusion results in a concomitant increase in secretion of cargo receptors via EVs.

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POSTER NUMBER: 3019

HUWE1 E3 ligase promotes PINK1/PARKIN-independent mitophagy by regulating AMBRA1 activation via IKK

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The selective removal of undesired or damaged mitochondria by autophagy, known as mitophagy, is crucial for cellular homeostasis, and prevents tumour diffusion, neurodegeneration and aging. The pro-autophagic molecule AMBRA1 (autophagy/beclin-1 regulator-1) has been defined as a novel regulator of mitophagy in both PINK1/PARKIN-dependent and -independent systems. Here, we identified the E3 ubiquitin ligase HUWE1 as a key inducing factor in AMBRA1-mediated mitophagy, a process that takes place independently of the main mitophagy receptors. Furthermore, we show that mitophagy function of AMBRA1 is post-translationally controlled, upon HUWE1-activity, by a positive phosphorylation on its serine 1014. This modification is mediated by the IKK kinase and induces structural changes in AMBRA1, thus promoting its interaction with LC3/GABARAP (mATG8) proteins and its mitophagic activity. Altogether, these results demonstrate that AMBRA1 regulates mitophagy through a novel pathway, in which HUWE1 and IKK are key factors, shedding new lights on the regulation of mitochondrial quality control and homeostasis in mammalian cells.

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POSTER NUMBER: 3020

Modulation of Autophagy by Mycobacterial PE/PPE proteins During Infection

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Autophagy has an essential role in both innate and adaptive immunity. Many microbes have evolved mechanisms to evade and/or exploit autophagy. *Mycobacterium tuberculosis* is well known to block phagolysosome fusion; however, it has been demonstrated that stimulation of the autophagic pathways causes mycobacterial phagosomes to mature into phagolysosomes. Thus, this induction of autophagy can suppress intracellular survival of mycobacteria. We hypothesize that mycobacterial virulence factors mediate autophagy evasion in order to ensure survival within the infected macrophages. The identification and characterization of such virulence factors will allow us to understand the mechanisms by which autophagy affects the outcome of host-microbe interactions and immune responses. Through loss-of-function screening of *M. tuberculosis* using transposon mutant screening, we were able to identify six of these regions contain genes belonging to the unique mycobacterial PE/PPE protein family. PE/PPE proteins are especially abundant in pathogenic mycobacteria and have been shown to play diverse roles in mycobacterial pathogenesis and in modulating critical innate immune pathways.

Using Yeast 2 Hybridization and co-immunoprecipitation we have identified host binding partners of these bacterial proteins during infection, which further evidences the modulation of autophagy by pathogenic mycobacteria. Increased knowledge of *M. tuberculosis* infection-induced autophagy presents an opportunity to uncover new and promising therapeutics and vaccines against tuberculosis to prevent mycobacterial infection and survival within the host.

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POSTER NUMBER: 3021

FAM134B oligomerization drives ER membrane scission for ER-phagy

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ER degradation by selective autophagy (ER-phagy) is crucial for ER homeostasis. However, it remains unclear how ER scission is regulated for subsequent autophagosomal sequestration and lysosomal degradation. Here, we show that oligomer formation of FAM134B through its Reticulon domain was required for membrane fragmentation in vitro and ER-phagy in vivo. Under ER stress conditions, cytoplasmic calcium concentration was elevated, which resulted in CAMK2B activation. Next, CAMK2B phosphorylated the Reticulon domain of FAM134B, which enhanced FAM134B oligomerization and activity in membrane fragmentation to accommodate high demand of ER-phagy. Unexpectedly, FAM134BG216R, a variant derived from a type II HSAN patient, exhibited gain-of-function defects, such as hyperactive in self-association and membrane scission, which resulted in excessive ER-phagy and sensory neuron death. Therefore, this study revealed a mechanism of ER membrane fragmentation in ER-phagy, along with a signaling pathway in regulating ER turnover, and suggested a potential implication of excessive selective autophagy in human diseases.

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POSTER NUMBER: 3022

Profiling the lysophagy pathway through quantitative proteomics

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Lysosomes are organelles that function at the terminal end of the endocytic, phagocytic, and autophagic pathways. Lysosomes maintain cellular homeostasis and quality control through nutrient and energy sensing pathways and the recycling of macromolecules and organelles. Therefore, it is essential for every cell to maintain a functional population of lysosomes which are subject to diverse physiological insults that rupture the lysosomal membrane such as oxidative stress, protein aggregates, or pathogens. Additionally, lysosomal membrane integrity is compromised in several pathophysiological conditions including prion disease and frontotemporal lobe disorder.

The consequences of lysosomal membrane permeabilization, if uncorrected, results in inflammation and cell death due to the release of reactive oxygen species, calcium, and cathepsins into the cytosol that damage cellular components.

The cell responds to lysosomal membrane damage through the selective autophagic recycling of these organelles (lysophagy). This sequential process includes the sensing of damaged lysosomes by galectins, their tagging by ubiquitin, recruitment of autophagic receptors, initiation of the autophagic program, and finally fusion of autophagosome-engulfed damaged lysosomes with healthy lysosomes. We have used an organelle enrichment strategy in combination with various forms of quantitative mass spectrometry (LFQ, TMT) in an attempt to both identify novel components of lysophagy and profile the ubiquitin chain linkages of damaged lysosomes. The lysosomal membrane protein TMEM192 was epitope (3xHA) tagged on its cytosolic C-terminus in HeLa cells using the CRISPR/Cas9 system. These cells were subsequently treated with the lysomotropic compound Gly-Phe β -naphthylamide to induce lysophagy and lysosomes were immuno-purified and subjected to quantitative mass spectrometry. We anticipate that our study will facilitate our understanding of the biology of lysophagy and provide novel targets for therapeutic intervention in disease states.

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POSTER NUMBER: 3023

The HaloTag-LC3 autophagosome completion identifies ESCRT subunits as regulators of phagophore closure

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The mechanism of phagophore closure remains unclear due to technical limitations in distinguishing unclosed and closed autophagosomal membranes. Here, we report the HaloTag-LC3 autophagosome completion assay that specifically detects phagophores, nascent autophagosomes, and mature autophagic structures. Using this assay, we perform focused and genome-wide library screens and identify several endosomal sorting complexes required for transport (ESCRT) components including the ESCRT-III CHMP2A as critical regulators of phagophore closure. Upon autophagosome formation, CHMP2A translocates to the phagophore and regulates the separation of the inner and outer autophagosomal membranes in a manner that is dependent on the AAA-ATPase VPS4 activity. Consistently, the phagophore recruitment of VPS4 requires the upstream ESCRT subunits. The ESCRT-mediated membrane abscission appears to be a critical step in forming functional autolysosomes by preventing mislocalization of lysosome-associated membrane glycoprotein 1 to the inner autophagosomal membrane. Collectively, our work reveals a function for the ESCRT machinery in the final step of autophagosome formation and provides a robust and high-throughput screen-compatible tool for the analysis of autophagosome completion.

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POSTER NUMBER: 3024

Rab3GAP-Rab18 module: a novel regulator of autolysosome maturation

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Autophagy is a conserved lysosomal degradation pathway in eukaryotes. During autophagy dispensable or aberrant organelles are sequestered into double-membraned autophagosomes that eventually fuse with lysosomes. Inside the newly formed autolysosomal lumen, the cargo is degraded and the resulting monomers are subsequently recycled. Thus, this process is essential for cellular homeostasis and autophagy dysfunction is linked to several diseases such as neurodegeneration, cancer and aging.

Autophagosome-lysosome fusion is a key step of autophagy, however our knowledge about its genetic regulation is

still limited. During the autophagic process, the cellular endomembrane system undergoes extensive transformation. Small GTPases of the Rab protein family are major regulators of membrane dynamics. The nucleotide bound state of Rab proteins is dependent of several cofactors, including the activator GEFs (guanine nucleotide exchange factor) and inactivator GAPs (GTPase-activating protein). Our research focuses on the complex of Rab3GAP2 and Rab3GAP1 proteins. The Rab3GAP1/2 complex is a multi-modal Rab cofactor as on one hand it can function as a GAP for Rab3, but on the other hand it can also act as a GEF for Rab18. In our study we found that the loss of Rab3GAP2 inhibits autophagic degradation in *Drosophila melanogaster*. In addition, we showed that loss of Rab3GAP2 leads to the severe alteration of lysosome morphology and function associated with autophagosome accumulation. Knocking down Rab18 inhibits autophagy in a similar way to Rab3GAP2. Finally, we found that aged Rab3GAP2 mutants show neuromuscular dysfunction, which is a well-known consequence of an autophagy defect. Taken together, our results suggest that the Rab3GAP-Rab18 module acts as a novel regulator of autolysosome maturation.

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POSTER NUMBER: 3059

Probing Mechanisms of Selective Autophagy Relevant to Aging

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Since autophagy is critical for development, disease, and aging, determining the molecular action of autophagy is crucial to better understand organismal health. Notably, autophagy is increasingly appreciated as a selective process by which specific types of cargo, such as mitochondria and lipids, are sequestered for lysosomal degradation via selective autophagy receptors, which simultaneously bind specific cargo and the autophagy protein Atg8/LC3/GABARAP. While accumulating evidence supports that disruptions in selective types of autophagy can contribute to the development of age-related disease, the relevant selective autophagy receptors and their role in aging remain understudied.

To identify novel selective autophagy receptors which may be relevant to aging, I have performed a candidate RNA interference (RNAi) screen using the short-lived and genetically tractable nematode *C. elegans* expressing GFP-tagged Atg8/LGG-1. In this primary screen, I have so far identified ~100 candidate genes to affect autophagy function. Furthermore, I found that ~50 of these candidate genes are conserved in mammals. I am now performing secondary RNAi screens utilizing cargo-specific reporters to search amongst these genes for potential autophagy receptors. Moreover, I plan to probe for interactions with Atg8/LGG-1 using immunoprecipitation and immunohistochemistry assays to verify such functions, and test for possible health- and lifespan roles of such candidate receptors.

This study will improve our molecular understanding of the regulation of autophagy and of the physiological relevance of autophagy receptors in aging. Such insights may provide possible new entry points for designing targeted therapeutic interventions against age-related diseases.

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POSTER NUMBER: 3025

Atg2A localization to mitochondria-associated ER membrane is required for phagophore elongation to control autophagosome size

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Mammalian Atg2A and Atg2B are functionally redundant in autophagy, but their precise roles in autophagosome biogenesis remain unclear. In this study, we utilize the newly developed HaloTag-LC3 autophagosome completion assay combined with TEM and CLEM to demonstrate the importance of human Atg2 proteins in autophagosomal membrane elongation. In Atg2A/B deficient cells, autophagosomes can still form and fuse with lysosomes, but the size of autophagosomes is significantly smaller than normal, which can be fully rescued by expression of Atg2A.

During autophagy, Atg2A translocates to phagophore formation sites on the mitochondria-associated ER membrane (MAM) through a short C-terminal region named MAM localization domain (MLD). Importantly, deletion or mutations in MLD abolishes the ability of Atg2A to localize to MAM and to rescue the autophagosome size in Atg2A/B deficient cells. By using tandem affinity purification and proteomic analysis, we identify a novel Atg2A interacting partner that is responsible for targeting Atg2A to MAM via MLD binding for phagophore elongation. Moreover, we demonstrate that Atg2A interacts with Atg9A through its N-terminus and loss of Atg2A/B causes accumulation of LC3-positive vesicles in MAM. Noteworthy, Atg9A deficient cells also accumulate small size autophagosomes. Collectively, this study suggests that Atg2A translocates to MAM upon autophagy induction to facilitate Atg9A retrograde trafficking for phagophore growth into normal size autophagosomes.

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POSTER NUMBER: 3057

Using iPSC models to describe the role of autophagy during neuronal development and manifestation of neurodegenerative diseases

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Neurodegenerative Diseases such as Parkinson's Disease or ataxias have repeatedly been linked to dysregulation of proteostatic pathways. In particular, impairment of autophagy (e.g. by perturbing mutations of relevant genes) can give rise to conditions that favor the build-up of protein oligomers and aggregates, resulting in break-down of cellular stress responses and ultimately leading to cell death of the affected cell population. Our aim is to employ induced pluripotent stem cell (iPSC) technology and neuronal differentiation paradigms for assessing how proteostasis pathways (proteasome, autophagy) are differentially regulated during neuronal development and how especially autophagy and its subtypes contribute to manifestation of neurodegenerative diseases. We demonstrate that autophagy pathway components (LC3-II, p62/SQSTM1) are upregulated during neuronal differentiation from neural stem cells (lt-NES) to immature neurons while proteasomal components remain unchanged. Using Bafilomycin A treatment, we show that basic autophagic flux is continuously increasing during in vitro differentiation. Using iPSC-derived dopaminergic neurons from a Parkinson's Disease patient carrying the causative LRRK2 G2019S mutation we demonstrate that defects in autophagy-mediated turnover of mitochondria (mitophagy) manifest as early as at day 8 of differentiation. These data point to a vital role of autophagy regulation during early steps of neuronal development. Lastly, we tested the hypothesis that autophagy dysregulation also contributes to formation of neuronal intranuclear inclusions (NIIs) of mutant Ataxin-3 protein in an iPSC model of polyglutamine disease (spinocerebellar ataxia type 3; SCA3). Inhibition of autophagy by 3-MA resulted in a significant increase of NIIs as detected by fluorescence microscopy in the susceptible cell population. Intriguingly, the build-up of NIIs could be abated by pharmacological upregulation of autophagic flux. Our data indicate that iPSC-derived neural stem cells and their neuronal progeny provide a versatile experimental platform for assessing the role of autophagy regulation in cellular homeostasis during human neural development and disease.

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POSTER NUMBER: 3027

Live-cell CRISPR gene-essentiality assay identifies mechanisms by which cancer cells circumvent autophagy dependence

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Some reports have suggested that cancer cells differ markedly in their dependence on autophagy; other studies have concluded that few, if any, cancer cells are truly dependent on autophagy. We developed a novel CRISPR/Cas9 assay in combination with live cell imaging that can acutely measure the effects of knockout (KO) of essential autophagy genes on cell viability and compare the behavior of KO and WT cells in the same population within hours of gene editing, eliminating clonal bias. When we compare a panel of 12 core autophagy regulators to genes that are generally considered to be "essential" (e.g. RNA POLII, PCNA, eEF2) or "non-essential" (e.g. PTEN, GFP) for

tumor cell survival and growth, we find that in some human tumor cell lines, multiple autophagy genes behave like “essential” genes. In other cell lines, the same autophagy genes behave like “non-essential” genes. However, unlike cellular processes such as DNA replication or gene transcription and translation that achieve cell functions that cannot be handled any other way, even highly autophagy-dependent cancer cells can, if put under sufficient selective pressure, evolve ways to circumvent even complete inactivation of essential autophagy regulators. In particular, autophagy dependent cell lines that have lost ATG7 can no longer turnover their mitochondria by mitophagy but can compensate and maintain mitochondrial function by altering the rate of mitochondrial fusion. Additionally, ATG7^{-/-} clones from autophagy dependent cell lines have retarded proteasomal turnover but can circumvent this loss by increasing the pro-survival NRF2 signaling pathway, mechanistically mediated by a p62-KEAP1 dependent de-repression of NRF2. Our results not only indicate an increased dependence on NRF2 in autophagy dependent ATG7^{-/-} cells, but also suggest that patients that relapse on autophagy targeted therapeutics may benefit from subsequent NRF2 inhibitors.

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POSTER NUMBER: 3028

Impaired autophagy and loss of TFEB action induces cardiomyocyte apoptosis and contractile dysfunction

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Our laboratory demonstrated that cardiomyocytes overloaded with lipid, decreases nuclear transcription factor EB (TFEB), the master regulator of lysosomal metabolism, signalling and autophagy. Since lysosomal function is impaired in the obese and diabetic heart, we hypothesized that preserving TFEB content attenuates lipid overload-induced cardiomyocyte dysfunction. We used rat cardiomyofibroblasts (H9C2) incubated with polyunsaturated fatty acid (PUFA) and saturated FA (palmitate) to assess the effect of distinct FA on TFEB regulation and action. Immunoblot analysis revealed that unlike PUFA, palmitate augmented caspase-3 cleavage, decreased TFEB levels and lysosomal proteolytic activity in a concentration-dependent manner. Compared to chow diet, C57BL6J mice fed high-fat high-sucrose diet for 8, 12 and 16 weeks showed a decrease in nuclear TFEB content in the heart which paralleled cardiac dysfunction. Notably, contractility studies in electrically-paced ventricular cardiomyocytes from constitutive myocyte specific TFEB knock out mice revealed reduction in sarcomere length, maximal velocity of shortening and relengthening compared to WT cells independent of altered intracellular calcium transient. We next examined if restoring TFEB attenuates palmitate-induced cell death. Adenoviral-mediated overexpression of TFEB in neonatal rat cardiomyocytes (NRCMs), alleviated palmitate-induced caspase-3 cleavage and activity and reduction in cathepsin activity. Moreover, preserving TFEB action blunted palmitate-induced insulin resistance in NRCMs by enhancing insulin-mediated AKT phosphorylation compared to control group. Our findings signify that increased TFEB levels likely protects against lipid overload by modulating insulin-mediated metabolism and survival. To conclude, restoring TFEB action during nutrient overload attenuates lysosomal dysfunction and cell death, likely by sensitizing the cardiomyocyte to insulin-signalling thereby improving cardiomyocyte function.

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POSTER NUMBER: 3029

Targetome Analysis of Chaperone-Mediated Autophagy in Cancer Cells

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Chaperone-mediated autophagy is a lysosomal degradation pathway of select proteins. Although, one-third of the soluble proteins are predicted to be recognized by this pathway, only a minor fraction of its proteome has been identified as CMA substrates in cancer cells. Furthermore, it is unclear whether groups of proteins with similar functions or proteins assigned to specific and well-defined cellular processes are selectively targeted for degradation by CMA in different physiological or pathological settings. Here, we undertook a quantitative multiplex mass spectrometry approach to study the proteome of isolated lysosomes in cancer cells during CMA activated conditions. By integrating bioinformatics analyses, we identified and categorized proteins of multiple cellular

pathways that were specifically targeted by CMA. Beyond verifying metabolic pathways, we show that multiple components involved in select biological processes, including cellular translation, is specifically targeted for degradation by CMA. In particular, several proteins of the translation initiation complex were identified as *bona fide* CMA substrates in multiple cancer cell lines of distinct origin and we show that CMA suppresses cellular translation. We further show that the identified CMA substrates display high expression in multiple primary cancers compared to their normal counterparts. Combined, our findings not only uncover specific cellular processes affected by CMA but also show the selectivity of CMA in targeting select biological processes in cancer cells and reveal a novel role for CMA in the control of translation initiation.

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POSTER NUMBER: 3030

Spatiotemporal control of ULK1 activation by NDP52 and TBK1 during selective autophagy

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Selective autophagy is degradative process that allows for the specific clearance of damaged organelles or intracellular pathogens. The ULK1 kinase drives the process of autophagosome biogenesis. However, it is unknown how ULK1 is activated during selective autophagy. In contrast, ULK1 activation during starvation-induced autophagy has been shown to be regulated by mTOR and AMPK kinases, two central energy signaling hubs. Here, we used chemically-inducible protein localization assays (CIDs) and CRISPR/Cas9 gene editing to dissect the mechanism of selective autophagy initiation. We demonstrate that ectopic localization of NDP52 to either mitochondria or peroxisome is sufficient to initiate selective autophagy by localizing and activating the ULK1 complex, leading to organelle degradation. NDP52 is able to initiate selective autophagy by interacting with FIP200 and this interaction in turn is mediated by TBK1 kinase. The ability of NDP52 to initiate selective autophagy depends on its interaction with the ULK1 complex, but not with LC3/GABARAP family proteins. Importantly, the autoactivation of ULK1 after cargo localization by NDP52 and TBK1 occurs independently of AMPK. Finally, ectopic localization of ULK1 to cargo bypasses the requirement for both NDP52 and TBK1 in initiating selective autophagy. Our findings delineate a novel link between cargo recognition and the autophagy initiation machinery. We thus provide a model of selective autophagy which highlights the coordination of ULK1 complex localization by NDP52 and TBK1 as principal drivers of targeted autophagosome biogenesis.

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POSTER NUMBER: 1004

Hermes, a monocarboxylate transporter that is necessary for autophagy during cell death

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Macroautophagy (autophagy) is a conserved process fundamental to all eukaryotic cells. It is a key catabolic process that is regulated by the nutrient sensor mechanistic target of rapamycin (mTOR) and is an important node in the metabolic network of all cells. Various stimuli, such as metabolic changes, hypoxia and hormones induce autophagy. Studies in animals indicate that autophagy possesses complex regulatory mechanisms and functions under different cell contexts. While much is known about autophagy's role in cell homeostasis, nutrient sensing and cell survival, less is known about its physiological role during programmed cell death.

Our lab has previously shown that autophagy participates in developmentally programmed cell death of *Drosophila* larval salivary glands. By performing a candidate-based genetic screen, we identified a monocarboxylate transporter, named Hermes, that influences autophagic cell death in *Drosophila* salivary glands, but it is not

required for either developmentally programmed autophagy in the larval midgut or for starvation induced autophagy in the fat-body of *Drosophila*. We show that Hermes transports pyruvate and affects autophagic cell death in an mTOR dependent manner. Importantly, Hermes' human homologue, SLC16A11, also a pyruvate carrier, was recently identified as a type 2 diabetes risk gene. The relationship of this gene to proper regulation of autophagy during cell death will be presented.

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POSTER NUMBER: 3033

A novel role of autophagy in coordinating meiosis exit and sporulation in *Saccharomyces cerevisiae*

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Meiosis is an essential process for gametogenesis, the production of cells specialized in sexual reproduction. It must be coordinated with cytokinesis so that closure of the daughter cell membrane occurs only after nuclear division is complete. Autophagy was generally believed to be required for entering meiosis but downregulated and nonessential during meiosis. However, my laboratory recently optimized an autophagy reporter system, which when coupled with synchronized meiosis in the budding yeast (*Saccharomyces cerevisiae*) led to the discoveries that (a) autophagy levels oscillate during meiosis; (b) loss of meiotic autophagic activity causes a novel meiotic phenotype, i.e. "Meiosis Defective Exit (DE)", which features extra rounds of Spindle Pole Body (SPB, the yeast centrosome) replication and chromosome segregation, resulting in defective meiosis exit and prospore membrane closure (i.e. cytokinesis). We further discovered that, at the onset of meiosis II, autophagy rapidly clears amyloid-like Rim4, an essential meiotic prion-containing RNA binding protein (RBP), which was reported forming amyloid-like aggregates to sequester and post-transcriptionally represses translation of mid-late meiotic mRNAs. Therefore, the accumulation/clearance of amyloid-like Rim4 controls temporal expression of mid-late meiotic proteins, e.g. Clb3, a reported B-type cyclin that is specifically required at meiosis II to regulate sister chromatid separation. Thus, autophagy is critically involved in meiosis progression and completion by stimulating translation of numerous transcripts (e.g. *CLB3*) that are sequestered by amyloid-like Rim4. In addition, we discovered that autophagy degrades prospore membrane (PSM) associated proteins (e.g. Spo74 and Don1) to allow membrane closure at the end of meiosis II, suggesting that autophagy coordinates meiosis II exit with PSM closure (cytokinesis). Our studies reveal a novel role of autophagy in yeast gametogenesis and link autophagy with amyloidogenesis, translational control of gene expression and subcellular compartment remodeling in the context of meiotic cell division.

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POSTER NUMBER: 3058

Molecular regulation of Atg9 under oxidative stress conditions

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Autophagy is a highly conserved catabolic process that degrades and recycles cellular components through lysosomes in response to various forms of stresses. Among the autophagy-related proteins, Atg9 is the only multipass transmembrane protein and is thought to act as a lipid carrier during autophagosome formation. But the molecular regulation and physiological function of Atg9 remain elusive. Our previous study showed that *Drosophila* Atg9 interacts with dTRAF2 to regulate JNK signaling and is important for the maintenance of midgut homeostasis under oxidative stress conditions. Here, we show that the mammalian dTRAF2 homolog TRAF6 mediates Atg9 ubiquitination and promotes its interaction with another autophagy initiator Beclin1 in response to H₂O₂ stimulation. Notably, the assembly of Beclin1-Vps34-Atg14L complex is markedly decreased in Atg9 or TRAF6 knockout cells, and results in the reduction of Vps34 activity. Depletion of TRAF6 decreases LC3 puncta formation and disrupts the colocalization of Atg9 with LC3 puncta upon H₂O₂ treatment. Our findings provide a mechanistic insight of Atg9 regulation under oxidative stress conditions.

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POSTER NUMBER: 3032

Requirement for autophagic lipid metabolism to sustain mTORC1 hyperactivation in TSC-deficient neural stem cells

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While mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) negatively regulates autophagy in cellular homeostasis, much less is understood on how autophagy impacts on mTORC1 signaling in vivo. By creating and analyzing a mouse model with double conditional knockout of *Tsc1* and an essential autophagy gene *Fip200* in neural stem/progenitor cells (NSCs), we describe mechanisms by which autophagy controls mTORC1 hyper-activation and the neurodevelopmental lesions of Tuberous Sclerosis Complex (TSC) including defective NSC maintenance, differentiation and tumorigenesis. We show that TSC-deficient NSCs and other types of cells require autophagy to maintain mTORC1 hyper-activation under energy stress conditions. Autophagy of lipid droplets (i.e. lipophagy) is used as an alternative energy source to fuel OXPHOS, ATP generation, and mTORC1 during energy stress. In vivo, targeting lipophagy or its downstream catabolic pathway reverses defective phenotypes caused by *Tsc1*-null NSCs and blocks their tumorigenesis in mouse models. These results reveal a cooperative function of selective autophagy in coupling energy availability with TSC pathogenesis, providing potential new therapeutic strategies to benefit TSC and/or cancer patients driven by mTORC1 hyper-activation.

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POSTER NUMBER: 3034

Select autophagy genes prevent hyper-inflammation by maintaining quiescence of myeloid cells

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Autophagy, a pathway for lysosomal degradation of cytosolic materials, is important for immunity. Autophagy genes can have roles independent of degradative autophagy pathway. Here we show that mice lacking certain autophagy genes in myeloid cells exhibited enhanced resistance to *Listeria monocytogenes* due to proinflammatory macrophage activation present prior to infection. Autophagy genes limited to the ULK and PI3K complexes involved in initiating autophagy, namely *Becn1* (Beclin 1) and *Fip200*, but not genes in the ubiquitin-like conjugation systems of autophagy regulated spontaneous activation of tissue-resident macrophages and resistance to *Listeria*. The activity of these genes in macrophages prevented hyper-inflammation by keeping phagocytes immunologically quiescent at baseline. Transcriptome and single-cell RNA sequencing revealed that macrophages in mice lacking *Becn1* in myeloid cells exhibited elevated IFN γ signaling. The hyper-inflammatory macrophage phenotype, as well as secondary effects on B and T cell phenotypes, was dependent on the presence of the IFN γ receptor, but not on inflammasomes. IFN γ driving the hyper-inflammatory state was derived from T cells with innate cells playing a minor role. We observed diminished basal macrophage activation in antibiotic treated wild-type mice but not mice lacking *Becn1* in myeloid cells, establishing the dominance of autophagy gene regulation over effects of the microbiota in controlling the level of macrophage activation. These studies indicate that certain autophagy genes, but not the degradative autophagy pathway, are responsible for setting the level of myeloid cell activation in uninfected hosts dampening immune system activation in the host prior to challenge with an infecting agent.

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POSTER NUMBER: 3035

Clearance of cell corpses and cell debris by LC3-associated phagocytosis

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Cells use non-canonical forms of autophagy to clear cell corpses and cell fragments, including processes such as

LC3-associated phagocytosis (LAP) ¹. We have been studying the clearance of two native LAP cargos in *Caenorhabditis elegans* embryos, including a cell fragment derived from the final step of cytokinesis called the midbody remnant^{2,3} and a cell that undergoes programmed necrosis after fertilization called the polar body⁴. Exposure of the lipid phosphatidylserine on these cell corpses and cell debris recruits engulfment receptors to the plasma membrane. Receptor trafficking depends on the PI3Kinase VPS-34, RAB-5 GTPase, and the sorting nexin SNX-6. Receptor-mediated engulfment pathways converge on the Rac1 ortholog CED-10 to drive actin polymerization and extend the plasma membrane to engulf cell corpses and cell debris in a phagosome. Rab GTPases and LC3 homologs are sequentially recruited to the phagosome membrane, which also depends on the PI3Kinase VPS-34. RAB-7 GTPase is required for lysosome recruitment to the phagosome, while LC3 lipidation promotes degradation of the corpse membrane inside the phagolysosome. After membrane breakdown, the polar body phagolysosome tubulates into small vesicles in an mTOR- and ARL-8-dependent manner, which facilitates its timely degradation. These genetic model systems reveal insights into the mechanisms of phagocytosis, LC3-associated phagosome maturation and phagolysosomal degradation, expanding the repertoire of cargos degraded by non-canonical autophagy *in vivo*.

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POSTER NUMBER: 3036

The novel role of transcriptional factor Spt4-Spt5 complex in regulating autophagy

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Macroautophagy/autophagy, a highly conserved dynamic process, is one of the major degradative pathways in the cell. Autophagy plays a crucial role in cell survival and maintenance, and its dysfunction is related to various diseases, indicating that the proper regulation of autophagy is important. So far, more than 40 autophagy-related (ATG) genes have been identified in yeast, and most of the corresponding protein products are important factors that function to initiate, complete and regulate autophagy. Although the overall process of autophagy has been extensively studied, in particular with regard to the function of autophagy-related (Atg) proteins, relatively little is known about the complicated modulation of gene expression and specific transcriptional regulators of autophagy.

The success of transcription requires contributions from different transcription accessory factors, one of which is the heterodimeric Spt4-Spt5 complex. In higher eukaryotic cells, the Spt4-Spt5 complex plays a dual role in gene regulation, acting both to delay transcription through promoter-proximal pausing, and to facilitate transcriptional elongation. In contrast, in *S. cerevisiae*, only the positive function of the Spt4-Spt5 complex has been identified.

Here, we show that the Spt4-Spt5 complex negatively regulates ATG gene expression in *S. cerevisiae*, thereby inhibiting autophagy activity during active growth. We also find that Spt5 can directly bind to specific DNA regions downstream of transcription start sites of corresponding ATGs when the nutrient is rich. Under autophagy-inducing conditions, the repression on ATG expression is released upon the enhanced phosphorylation of Spt5, allowing an upregulation of autophagy activity. Taking all the data together, we show for the first time a negative function of the Spt4-Spt5 complex in *S. cerevisiae*, and these studies also advance our understanding of the fine-tuned regulation of autophagy at the transcriptional level.

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 POSTER NUMBER: 3037

Generation of mice defective in LC3-associated phagocytosis for studies of microbial infection

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Most pathogens enter cells by endocytosis or phagocytosis. Recent work has identified a pathway called LC3-associated phagocytosis (LAP) that targets endosomes and phagosomes containing pathogens as they enter cells. LAP results in the recruitment of autophagy protein LC3 to the phagosome/endosome membrane and this may enhance delivery of pathogens to lysosomes for degradation. The recruitment of LC3 to membranes requires autophagy protein ATG16L1. ATG16L1 in mammals contains an N-terminal ATG5-binding domain and a coiled coil domain (CCD) attached to a larger C-terminal WD repeat domain. The observation that the WD domain is required for LAP 'in vitro' (1) prompted us to generate mice (WD) lacking the WD domain of ATG16L1 to study the roles played by LAP 'in vivo'. The WD mice grew at the same rate as littermate controls, and were fertile. Analysis of bone marrow derived macrophages and dendritic cells showed that the WD mice cannot activate LAP (LAP^{-/-}). Pathology and histology screens failed to identify defects in the major tissues of the LAP^{-/-} mice suggesting that LAP is not required for tissue homeostasis in mice 'in vivo' (2). The LAP^{-/-} have been challenged with influenza virus and salmonella to determine if LAP evolved to protect against microbial infection.

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 POSTER NUMBER: 3038

Synaptic Autophagy

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Autophagy is an essential cellular process for degrading damaged proteins and organelles, but little is known about its function at synapses. In neurons, autophagosomes are readily produced in axons and transported to the cell body for fusion with lysosomes, where their cargo is degraded. Despite previous reports that lysosomes are largely excluded from the axon, we detect acidified degradative structures—presumably autolysosomes—at synapses in wild-type *C.elegans* expressing a tandem-fluorescent mCherry-eGFP-LC3 marker, which is yellow in neutral pH autophagosomes and red in acidic autolysosomes. The presence of SNAP proteins regulates the number and size of these structures. In the absence of SNAP-25, an essential protein for synaptic exocytosis, these lysosomes proliferate in the axon and become much larger. This phenotype can be rescued by overexpressing SNAP-25 or its homolog SNAP-29. We are currently characterizing these degradative structures and investigating the contribution of SNAP-25 and SNAP-29 to synaptic autophagy.

 POSTER NUMBER: 3039

Targeting of protein aggregates to lysosomes and implication for neurodegenerative diseases

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The accumulation of protein aggregates is involved in the onset of many neurodegenerative diseases. Aggrephagy is a selective type of autophagy that counteracts neurodegeneration by recycling such aggregates. The molecular machinery involved in targeting autophagic membranes to protein aggregates during autophagosome biogenesis has been well characterized. However, later steps of autophagy including targeting of autophagosomes to lysosomes are still not fully understood. Through a combination of classical biochemistry and cell biology with cutting edge in vitro reconstitutions, we have been able to discover a selective pathway that targets autophagosomes to lysosomes during aggrephagy. A dedicated set of autophagy proteins is involved in this pathway and their close cooperation is required to target autophagosomes to lysosomes for degradation. Importantly, neural cells are particularly sensitive to perturbations of this pathway, demonstrating that aggrephagy is essential to maintain neural homeostasis.

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POSTER NUMBER: 3040

Sex-dependent regulation of autophagy by midkine in pediatric dilated cardiomyopathy

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Background: Sex hormones are thought to influence sex-based clinical and molecular differences in adult patients with dilated cardiomyopathy (DCM). Interestingly, sex differences are also observed in pre-pubertal pediatric DCM patients, as girls with DCM have worse outcomes than boys. This suggests that sex-hormone-independent differences also play a role in cardiac pathology. One potential factor which may regulate sex differences in pediatric cardiac function is midkine (MDK), a protein that is highly elevated in the serum of pediatric DCM patients. While MDK's role in cardiac dysfunction is unclear, in cancer models, MDK has been found to inhibit autophagy. The objective of this study was to determine if high exogenous MDK leads to sex differences in autophagy and cardiac function.

Methods: Young male and female mice were treated for 7 days with 3mg/kg/week recombinant human MDK. Cardiac pathology was assessed by analysis of gene expression and protein changes, echocardiogram, and sarcomeric contractility and relaxation measured through myofibril mechanics.

Results: Female mice treated with MDK demonstrated upregulation of genes involved in the fetal gene program. Further, there were sex-specific differences in whole heart and subcellular function in MDK treated mice. Fractional shortening and rate of activation of contraction were decreased in female mice treated with MDK. In addition, myofibril relaxation mechanics were altered in female mice treated with MDK. Finally, analysis of mediators of autophagy indicate that MDK in female mice leads to inhibition of autophagy in the heart.

Conclusions: Elevated exogenous MDK in mice leads to sex-specific differences in cardiac function, sarcomeric mechanics, and alterations in autophagy. These findings suggest that MDK may contribute to worsened outcomes in pediatric girls with DCM.

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POSTER NUMBER: 3041

Identification of bleomycin target that drives fibrosis by hampering TFEB-mediated autophagy

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Bleomycin is a widely used cancer drug for the treatment of germ-cell tumors, lymphomas, cervical cancer and squamous-cell carcinomas. Bleomycin causes severe pulmonary fibrosis. The molecular target of bleomycin, remains unknown. Using a chemical proteomics approach, we identify ANXA2 (annexin A2) as a direct binding target of bleomycin with molecular interaction. Depletion of *anxa2* in mice mitigates bleomycin-induced pulmonary fibrosis. Glu139 (E139) of ANXA2 is required for bleomycin binding in lung epithelial cells. A CRISPR-Cas9-engineered ANXA2^{E139A} mutation in lung epithelial cells ablates bleomycin binding and activates TFEB (transcription factor EB), a master regulator of autophagy, leading to enhanced autophagy. Activation of TFEB elevates bleomycin-initiated autophagic flux, inhibits apoptosis and proliferation of epithelial cells, and ameliorates pulmonary fibrosis in bleomycin-treated mice. Lowered TFEB and LC3B levels were found in human pulmonary fibrosis tissues. Collectively, we identify ANXA2 as a specific bleomycin target that binds bleomycin to impede TFEB-induced

autophagy and thereby intensifying pulmonary fibrosis. Our data may provide insight into development of optimized bleomycin therapeutics devoid of lung toxicity by perturbing the ANXA2 binding.

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POSTER NUMBER: 3042

Sphingolipid Modulation Activates Autophagy and Proteostasis Programs to Govern Human Hematopoietic Stem Cell Self-renewal

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Cellular stress responses serve as crucial decision nodes to balance persistence or culling of hematopoietic stem cells (HSC) for lifelong blood production. While HSC culling mechanisms are known, the linkage between stress programs and self-renewal properties that underlie human HSC persistence remains unknown. Here, we provide the first direct evidence that sphingolipid composition regulates both HSC stress and self-renewal. We found that *ex vivo* culture of human HSC/progenitors (HSPC) alters the epigenetic landscape causing a loss of chromatin accessibility normally associated with dormant long-term (LT)-HSC, with resultant loss of functional LT-HSC. By contrast, inhibition of the sphingolipid enzyme DEGS1 specifically in HSC with *N*-(4-hydroxyphenyl) retinamide (4HPR) activates proteostatic quality control (QC) programs that coalesce around the unfolded protein response (UPR), an ATF4-regulated integrated stress response, and autophagy; the latter confirmed by immunofluorescence and flow cytometry in CB stem cells. These results suggest that ceramide, the central component to all sphingolipids, may act as a “lipid biostat” for measuring cellular stress and activating stress responses. We further asked if 4HPR could synergize with known compounds to enhance HSC self-renewal. Treatment of HSPC with a combination of 4HPR plus CD34⁺ agonists UM171 and StemRegenin-1 (SR1) during *ex vivo* culture maintained a chromatin state more similar to uncultured LT-HSC as demonstrated by ATAC-seq, and led to a 4-fold increase in serial repopulating ability with xenotransplant assays over treatment with UM171 and SR1 alone. This work identifies a new linkage between sphingolipid metabolism, proteostatic QC systems and HSC self-renewal, and identifies novel strategies by which to expand HSC numbers and improve HSC quality for clinical applications.

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POSTER NUMBER: 3043

Parkin/PINK1-independent Mitophagy: Molecular Identity and Role in Fatty Liver Disease

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Elimination of mitochondria via a selective form of autophagy, termed mitophagy, is an essential cellular process for maintaining mitochondrial health. We found that liver-specific knockout (KO) of Drp1, a dynamin-related GTPase that is required for mitochondrial division, generates enlarged megamitochondria. These megamitochondria blocked mitophagy in Drp1KO hepatocytes and heavily accumulated ubiquitin and LC3 on their surfaces. Although parkin/PINK1-mediated mitochondrial ubiquitination has been shown to induce mitophagy, we found that both parkin and PINK1 are dispensable for mitochondrial ubiquitination in Drp1KO hepatocytes. Surprisingly, we found that p62, which has been considered to be an autophagy adaptor protein downstream of ubiquitination, is required for mitochondrial ubiquitination in Drp1KO hepatocytes. p62 was found to be associated with the surface of megamitochondria and recruit the Keap1/Rbx1 ubiquitin E3 ligase complex via direct interactions with Keap1. Additional loss of Opa1, another dynamin-related GTPase that is required for mitochondrial fusion, re-established mitochondrial size in Drp1KO hepatocytes and rescued mitophagy defects caused by division deficiency. Therefore, p62/Keap1/Rbx1-mediated mitophagy requires the regulation of mitochondrial size, but not mitochondrial division.

It has been reported that human patients with non-alcoholic fatty liver disease (NAFLD) form megamitochondria in hepatocytes similar to Drp1KO hepatocytes. Using a mouse model for diet-induced NAFLD, we found that these megamitochondria are also defective in p62/Keap1/Rbx1-mediated mitophagy and accumulate ubiquitin on their surfaces. Similar to Drp1Opa1KO hepatocytes, the loss of Opa1 decreases the size of mitochondria and cleared ubiquitin from the mitochondria of the NAFLD hepatocytes. Importantly, the loss of Opa1 significantly mitigated liver damage in this mouse model. Our data suggest that parkin/PINK1-independent mitophagy plays an important

POSTER NUMBER: 3045

LC3 and GABARAP subfamilies of mouse ATG8 differentially down-regulate innate immunity

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Inflammasome is a cytosolic multiprotein complex that assembles in response to a variety of cellular stress, infection and toxin. Activation of this system induces pyroptosis and release of mature IL-1 β and IL-18 to activate innate immune responses. Recent studies showed that some autophagy proteins (ATGs) are involved in the regulation of inflammasome, consisting of canonical and non-canonical pathways.

Here, we found that mammalian ATG8 proteins consisting of LC3 and GABARAP subfamilies differently regulate canonical and non-canonical inflammasome. Although LC3 subfamily was important for the canonical inflammasome, GABARAP subfamily was dispensable. On the other hand, GABARAP but not LC3 subfamily was required for regulation of non-canonical inflammasome. In particular, Gabarapl2 (also known as Gate-16) is the most important for regulation of non-canonical inflammasome activation in the GABARAP subfamily. Furthermore, activation of non-canonical inflammasome by LPS transfection or treatment of bacterial outer membrane vesicles (OMVs) was strongly enhanced in Gabarap and Gate-16 double deficient cells.

Taken together, these results demonstrate that LC3 and GABARAP subfamilies differentially and negatively inflammasome activation in innate immunity.

POSTER NUMBER: 3044

The physiologic cargoes of macroautophagy in aging and disease

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Although macroautophagy, and the dysfunction thereof, is of fundamental interest across a wide array of diseases, little is known about the identity of those substrates that rely upon this degradation pathway for their turnover. Using a quantitative proteomic approach, we are characterizing the proteomic content of isolated autophagic vacuoles from brain, spinal cord and liver of mice. In addition to determining how classical stimuli such as physiologic starvation might influence the distribution of cargoes, we are also taking advantage of genetically modified mice to determine how mutations implicated in neurodegenerative diseases might impact cargo capture. Comparative analyses reveal distinct differences across tissues including those comparisons between brain and spinal cord, suggesting that mutations impacting different aspects of autophagy could lead to tissue-specific outcomes. Overall, these studies will determine whether monitoring autophagic cargoes might give insight into how changes in macroautophagy might differentially contribute to the wide array of disorders in which this pathway has been implicated.

POSTER NUMBER: 3046

The essential autophagy gene *Atg5* is required for intestinal homeostasis and survival of adult mice

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Autophagy captures intracellular components and delivers them to lysosomes for degradation and recycling. *Atg7* and *Atg5* are both essential autophagy genes involved in autophagosome formation and deleting these genes leads to autophagy deficiency. We reported previously that whole body *Atg7* deletion in adult mice causes liver and muscle

damage, depletion of white adipose tissue, and shortens life span to 3 months due to neurodegeneration. As essential autophagy genes can function differently, we sought to test if whole body *Atg5* deletion in adult mice would have similar phenotype to deletion of *Atg7*. Whole body *Atg5* Δ/Δ mice generated by acute deletion had a much shorter lifespan compared to *Atg7* Δ/Δ mice. *Atg5* Δ/Δ mice showed severe damage in the ileum part of intestine, with loss of villus structure and barrier function. In contrast to *Atg7* Δ/Δ mice, the ileum of *Atg5* Δ/Δ showed more rapid loss of *Atg5* and autophagy, decreased staining for the stem cell marker OLFM4 and a diffuse stain pattern for the Paneth cell marker Lysozyme, suggesting loss of stem cells and malfunction of Paneth cells. Furthermore, *Atg5* Δ/Δ mice showed a decreased level of active β -catenin in the ileum, which is the key transcription factor at the Wnt signaling pathway that is essential for intestinal stem cell renewal. *Atg5* Δ/Δ mice also showed decreased staining for the mesenchymal cell marker PDGFR α in the ileum, suggesting the loss of all the PDGFR α positive mesenchymal cells in the ileum, which is required for the activation of Wnt signaling pathway in the stem cells. These findings suggest that *Atg5* is essential for the maintenance of PDGFR α positive mesenchymal cells to contribute Wnt signals to the stem cells for the maintenance of intestinal homeostasis and adult mice survival. Deletion of *Atg5* more gradually overcame the loss of ileum stem cells and resulted in death instead from neurodegeneration similar to deletion of *Atg7* or *Atg12*. Thus, rapid loss of autophagy revealed its novel role in Wnt signaling and ileum stem cell maintenance.

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POSTER NUMBER: 3047

Structural basis of human Atg3 activation and regulation for LC3-PE conjugation in autophagy

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Autophagy is a highly conserved pathway that mediates the bulk degradation of intracellular components and plays a significant role in the progression and outcome of numerous diseases. A key step in autophagy involves the conjugation of protein LC3 to phosphatidylethanolamine (PE) in the membrane. Atg3 catalyzes this reaction, but to date how human Atg3 (hAtg3) is activated and regulated for the LC3-PE production remains largely unknown. The current Atg3 activation model was proposed based on a study of yeast Atg3 (yAtg3) by Nobel Laureate Dr. Ohsumi's group in 2013. Three residues were shown to be critical for its activity. We have examined their corresponding mutations in hAtg3. While hAtg3^{T244A} exhibits an effect similar to yAtg3^{T214A}, surprisingly, hAtg3^{F296S} and hAtg3^{H266L} demonstrate opposite effects as those observed for yAtg3^{F293S} and yAtg3^{H236L}, respectively. These results indicate that the molecular mechanism for hAtg3 activation may be different than that of yAtg3. In addition, we have found that the interaction of hAtg3 with highly curved membranes may play a critical role in its activation. We will present our study on understanding the molecular basis of hAtg3 activation and regulation for LC3-PE conjugation using a multidisciplinary approach including structural and biochemical analyses, mutagenesis, and in cell functional assays.

Reference

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POSTER NUMBER: 3048

Exploring the translational regulation of nitrogen-starvation induced autophagy in the yeast *Saccharomyces cerevisiae*

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Macroautophagy/autophagy defines an essential catabolic process in eukaryotic cells that targets a wide variety of cytoplasmic contents for lysosomal degradation. Autophagy is normally kept at basal levels to maintain cellular homeostasis, but it also can be rapidly augmented as a survival mechanism in response to stress signals such as starvation. Either too much or too little autophagy can be deleterious.

The process of autophagy from initiation to closure is tightly executed and controlled by the concerted action of autophagy-related (Atg) proteins, which exhibit marked homology from yeast to human. Regulation of autophagy

depends in part on regulating the expression of *ATG* genes. A tremendous amount of research has focused on transcriptional and post-translational regulation of *ATG*/Atg genes/proteins, but little is known about the translational control of autophagy. During starvation, the global translation is downregulated, whereas expression of most *ATG* genes is highly upregulated to support autophagy. This raises an intriguing question: is there a specific translational upregulation for *ATG* mRNAs?

Previous translome studies have reported increased translation efficiency for some *ATG* genes, suggesting the existence of translational control for autophagy. Considering that translational regulation frequently occurs through the action of RNA-binding proteins (RBPs), we performed a screen of RBPs using yeast as the model system, and the Atg1 level as the readout. We identified an RGG-motif protein, a type of RBP that is characterized by the presence of multiple Arg-Gly-Gly- and Arg-Gly-X- repeats and known to regulate translation, which is required for efficient translation of Atg1 and autophagy activity during prolonged nitrogen starvation. This protein can directly associate with *ATG1* mRNA under nitrogen-starvation conditions and promotes its interaction with the translation initiation complex. This mechanism acts to facilitate a bypass of the general translation repression for Atg1 to support the increased demand for autophagy activity.

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POSTER NUMBER: 3049

Mammalian ULK1 Complex Assembly

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The ULK1 complex is responsible for initiating the pre-autophagosome structure. The structures and mechanisms of action of the four subunit complex consisting of FIP200, ULK1, ATG13 and ATG101 are only known in a fragmentary way, limiting our understanding of autophagy biogenesis in mammals. Structural studies are restricted to truncated ATG13/101 HORMA dimer and the ULK1 kinase domain. Almost no structural information is currently available on FIP200, which is responsible for the largest portion of the ULK1 complex (~1500 residues). To better understand the ULK1 complex, our work focuses on purification, *in vitro* reconstitution and structural analysis of the ULK1 complex. A major challenge in studying the ULK1 complex is efficient purification, which is complicated by the size (~3400 amino acids in total) and nature of the ULK1 complex. We have been able to generate and purify intact recombinant ULK1 complex from a mammalian expression system. Hydrogen/deuterium exchange mass spectrometry and negative stain electron microscopy have been utilized to comprehensively map the domain assembly of the ULK1 complex. The structural data are interpreted in terms of new hypotheses for initiation of selective and bulk autophagy.

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POSTER NUMBER: 3050

Rubicon modulates bone formation negatively

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Rubicon negatively regulates the fusion between autophagosomes and lysosomes. However, the physiological roles of Rubicon remain elusive *in vivo*. We now investigate the role of Rubicon in bones by generating osteoblast specific Rubicon deficient mice. We generated conditional knock out mice {Osterix-Cre/Rubicon (flox/flox)} that lack Rubicon in osteoblast. Osterix-Cre/Rubicon (flox/flox) mice progressively developed skeletal abnormalities in femur bones. In addition, Rubicon deficient osteoblast exhibited increased differentiation, mineralization and expression levels of transcription factors, such as *Runx2* and *Osteocalcin* that are master regulators of osteoblast.

Furthermore, Notch, negative regulator of osteoblast, signaling downstream transcription factors such as *HeyL*, *Hes1* and *Hes3* were expressed at lower levels in Rubicon deficient osteoblast. Consistently, we found reduction the protein expression of Notch receptor due to upregulation of autophagic flux in Rubicon deficient osteoblast. These data suggested that Notch receptor could be degraded by autophagy or reduced by other mechanisms. We are now generating physiological mice model such as a bone defect model (surgically creating a drill-hole into the bone) and an ovariectomy (removal of ovary) model using Osterix-Cre/Rubicon (flox/flox) mice to investigate

beneficial effects of Rubicon deficiency. These trials are valuable to confirm that Rubicon inhibitors have possibilities to be new therapeutic targets, such as osteoporosis and bone fracture.

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POSTER NUMBER: 3051

ATG2 is a lipid transporter at ER-autophagosome contacts during autophagosome biogenesis

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Despite decades of efforts in uncovering the identity and functions of molecules involved in autophagosome biogenesis, how lipids are delivered to the growing isolation membrane remains a key unanswered question in the field. Here we show that the autophagy-related (ATG) protein ATG2A is a lipid transport protein which localizes at ER-autophagic membrane contact sites to regulate autophagosome biogenesis. Human ATG2 shares secondary structure similarity with a recently identified lipid transport family VPS13. We found that ATG2 binds tens of glycerophospholipids and that when tethered at membrane interfaces *in vitro*, ATG2A transfers lipids between liposomes as efficiently as other established lipid transfer proteins. *ATG2A/ATG2B* double knockout (DKO) HEK293 cells are completely defective in autophagic degradation, and accumulate aberrant autophagosomal membranes, indicating dysregulation in membrane growth. Intriguingly, a minimal lipid transfer module in ATG2A, which efficiently transfers lipids *in vitro*, is both necessary and sufficient to restore autophagy in DKO cells. The rescue capacity is comparable to full-length ATG2A rescue, strongly suggesting that lipid transport is a central component of ATG2A function in proper autophagosome formation. With three color time-lapse imaging, we found that GFP-ATG2A associated with autophagosome puncta (BFP-LC3B) also localizes at the ER (RFP-Sec61b), and always demarcates the contact site between the ER and growing autophagosomes. Finally, by pre-clearing commercial anti-ATG2A antibodies with fixed DKO cells, we confirmed that endogenous ATG2A also localizes to ER-autophagic membrane contact sites. In summary, we found that nonvesicular lipid transport by ATG2 at membrane contact sites may be a principal mechanism for autophagosomal membrane expansion. This work for the first time depicts a clear mechanistic function for ATG2, and opens new avenues for future investigation of how autophagosomes grow.

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POSTER NUMBER: 3060

LAIP promotes autophagosome-lysosome fusion via interacting with Stx17 and HOPS complex

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Autophagy maintains cellular homeostasis by delivering damaged organelles and large molecules to the lysosome for degradation and reuse, and plays critical roles in cancer development. Autophagosome-lysosome fusion is mainly mediated by Stx17/SNAP29/Vamp8 interaction and the HOPS complex. However, the mechanism is still incomplete. We previously identified LAIP (LRP6-Axin-Interacting-Protein) as a potential Wnt signaling regulator (unpublished). LAIP is an intracellular protein that is enriched on the endosome and lysosome. ShRNA-mediated Knockdown or CRISPR-Cas9-mediated knockout of LAIP in cancer cells (such as HeLa and HCT116) affected cell growth and caused the accumulation of LC3II (autophagosome). Furthermore, LAIP interacts with Stx17 and the HOPS complex in the co-IP experiments. These results suggest that LAIP may play a role in autophagy and cancer development. We are currently further investigating the detailed mechanism and have generated the LAIP knockout mice to study its *in vivo* function.

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POSTER NUMBER: 3054

Beclin1 coiled coil domain distinctively regulates its interaction with Atg14L and UVRAG and can be targeted to

enhance autophagy and endolysosomal trafficking

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The Beclin1-Vps34 Class III PI3K complex is an essential module of the autophagy molecular machinery. Beclin1 is a scaffolding protein within this complex and binds to two mutually exclusive autophagy regulators Atg14L and UVRAG through its coiled coil domain to form biochemically and functionally distinct Atg14L- and UVRAG-containing Beclin1-Vps34 complexes. The potent and specific interaction between Beclin1 and Atg14L/UVRAG is of key importance to autophagy regulation but its molecular mechanism is not fully understood.

Our structural and biochemical study of the Beclin1 coiled coil domain reveals that it forms a homodimer destabilized by charged and polar residues at an otherwise hydrophobic dimer interface. Atg14L and UVRAG disrupt Beclin1 homodimer and form heterodimeric Beclin1-Atg14L/UVRAG complex to promote Vps34-dependent autophagy. On the other hand, mutations that stabilize the Beclin1 homodimeric state would reduce its association with Atg14L/UVRAG and inhibit autophagy. These results confirm that the metastability of Beclin1 coiled coil domain is critical for its interaction with Atg14L/UVRAG to regulate autophagy (*Nat. Comm.* 2012).

Furthermore, we also determined the crystal structure of the Beclin1-UVRAG coiled coil complex and identified an interface more strengthened than that for Beclin1 because of hydrophobic pairings and electrostatically complementary interactions. Potent Beclin1-UVRAG interaction is functionally significant for endolysosomal trafficking because it renders UVRAG more competitive than Atg14L in terms of Beclin1 binding. We also designed a few all-hydrocarbon stapled peptides that specifically targeted the C-terminal part of the Beclin1 coiled coil domain to interfere with its homodimerization. These peptides reduced Beclin 1 self-association, promoted Beclin1-Atg14L/UVRAG interaction, increased autophagic flux, and enhanced EGFR degradation (*PNAS* 2018). One such peptide even exerted potent anti-proliferative effect on a few cancer cell lines in non-apoptotic manner, suggesting a possible functional connection between autophagy and cell death.

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POSTER NUMBER: 3055

GAPR-1 inhibits Class III PI 3-Kinase complexes during autophagy induction

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Autophagy is an essential cellular process to maintain homeostasis, through which damaged and/or unwanted proteins, organelles and intracellular pathogens are enclosed by autophagosomes and degraded in autolysosomes. Class III PI 3-Kinase complexes (PI3KC3) consist of Vps34, Vps15, Beclin 1, and Atg14 (PI3KC3-C1) or UVRAG (PI3KC3-C2), and mediate autophagosome initiation and maturation, respectively. Previously we reported GAPR-1 as a novel Beclin 1 binding protein and showed that residues in an evolutionarily conserved groove of GAPR-1 are required for this binding (Shoji-Kawata et al., 2013; Li et al., 2017). Here we show that GAPR-1 not only binds to purified PI3KC3 but also inhibits the lipid kinase activity of PI3KC3 *in vitro*. The inhibition is likely due to GAPR-1 binding to a region in Beclin 1 adjacent to its membrane targeting domain, leading to a conformational change of PI3KC3 (Rostislavleva et al., 2015). GAPR-1 knockout (KO) in cells increases Beclin 1-associated Vps34 lipid kinase activity and recruits more PI3P-binding protein WIPI2 which functions in autophagosome formation. GAPR-1 KO cells and GAPR-1 KO mice have increased basal autophagic flux. Taken together, GAPR-1 is a negative regulator of autophagy and inhibits Class III PI 3-Kinase complexes during autophagy induction.

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POSTER NUMBER: 3053

Role of WDR45B in maintaining neural autophagy and cognitive function in mice

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The phosphoinositide PI(3)P effector Atg18 is essential for autophagosome formation in yeast. Mammalian cells contain four Atg18 homologs, WIPI1-4 (WD40 repeat protein interacting with phosphoinositides), which belong to two subclasses, WIPI1/2 and WIPI3/WIPI4 (known as WDR45/45B in humans). Recent human genetic studies have revealed a potential causative role of *WDR45B* in intellectual disability (ID). The role of *Wdr45b* in autophagy and in neuronal homeostasis, however, remains unknown. Here we demonstrated that mice deficient in *Wdr45b* exhibit motor deficits and learning and memory defects. Histological analysis reveals that *Wdr45* KO mice exhibit a large number of swollen axons and cerebellar atrophy. Autophagy substrates, SQSTM1 aggregates and ubiquitin-positive aggregates, accumulate in various brain regions, but not in other tissues in *Wdr45b* KO mice. *Wdr45/45b* double KO mice exhibit more severe autophagy defects than either single KO mouse and die within one day after birth, indicating that these two genes act redundantly in autophagy. Our studies demonstrated that WDR45B is critical for neuronal development and the knockout mice provide a model to study the pathogenesis of the related human disease.

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POSTER NUMBER: 3052

The Role of HERC1 in Autophagy Regulation

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HERC1 is a member of E3 ubiquitin ligases that contains three major domains: two RCC1-like domains and a C-terminal catalytic HECT domain. Even though HERC1 was first described more than 20 years ago, little is known. It is closely related to cell proliferation, energy sensing and cell membrane trafficking, but mechanism of HERC1 function is still not clearly characterized. Loss of function HERC1 mutations have been associated to a rare neurodevelopmental disorder (Macrocephaly, dysmorphic facies, and psychomotor retardation) characterized by large head and overgrowth. In addition, loss of function of HERC1 has been identified in a variety of cancer types. Although these mutations are not classified as cancer driver mutations, the mysterious connection between HERC1 and multiple signaling pathways provides exciting evidence for new drug target development. HERC1 is reported to regulate the mechanistic target of rapamycin complex 1 (mTORC1) by degrading both c-Raf and TSC2 in a ubiquitin dependent manner, altering cell proliferation and autophagy. Interestingly, high expression level of wild type HERC1 has been proved to favorably support the survival of cancer patients.

We decided to study the role of HERC1 on autophagy pathway in cancer cells. Autophagy is a well-regulated degradation pathway which cells use to recycle cellular contents to maintain homeostasis and viability during nutrient depletion. Autophagy-deficient tumor cells are more resistant to chemotherapy or T cell induced apoptosis and earlier reoccurrences. Given that multiple interactors that have been documented for HERC1, the precise mechanism underlying dysregulated autophagy in HERC1 mutant cells remains to be elucidated, with multiple models implicating mTOR as a key factor in HERC1-autophagy signaling axis. We developed different cell models with knock-out of HERC1 as well as overexpression of HERC1. Functional analysis of the mTOR and autophagy pathways revealed that HERC1 knock-out cells have decreased autophagy activation during starvation and more rapid cell growth in nutrient rich conditions. Loss of HERC1 targets, such as TSC2, also affects autophagy activity in our cancer cell model. The intriguing similar phenotype of HERC1 mutant and chemo-resistant cancer cells may lead to an innovative direction for cancer therapy.

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